MICROBES BY THE MILLION

by HUGH NICOL

THE AUTHOR

Dr. Nicol was born in London and is forty, "with all that implies." He matriculated in 1914 at Wood Green County School. He first became an accountant's clerk and later assistant chemist in a soap and glue works, and biochemist in hospitals for nervous diseases. He came to Rothamsted Experimental Station in 1930 as Assistant Bacteriologist. Throughout this time he pursued chemical studies in the evenings at institutions in London, and took successive degrees unaided by scholarships, which, he says, he was "too poor to afford." He is interested in languages and travel and is the author of *Plant Growth-Substances: Their Chemistry and Application.*

PUBLISHER'S NOTE

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MICROBES
BY THE
MILLION

BY

HUGH NICOL

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TO DAUGHTER BARBARA

WHO BECAME " PENGUIN "-CONSCIOUS

AT A VERY EARLY AGE

I WAS formerly a bookseller and binder, but am now turned philosopher,[1] which happened thus: Whilst an apprentice, I, for amusement, learnt a little chemistry and other parts of philosophy, and felt an eager desire to proceed in that way further. After being a journeyman . . . I gave up my business, and through the interest of a Sir H. Davy, filled the situation of chemical assistant to the Royal Institution of Great Britain, in which office I now remain; and where I am constantly employed in observing the works of Nature, and tracing the manner in which she directs the order and arrangement of the world.

—*Letter from Michael Faraday, taken from "Faraday as a Discoverer," by John Tyndall.* (*Longmans: London*, 1877.)

[1] Faraday loved this word and employed it to the last; he had an intense dislike to the modern term *physicist*. [J.T.]

CONTENTS

CHAPTER		PAGE
I.	A Saltspoonful of Soil	11
	Interlude	19
II.	What Microbes are:	
	(*a*) Fungi (Yeasts, Moulds, Mushrooms, etc.)	21
	(*b*) Protozoa, and something about Measurements	27
	(*c*) Bacteria	31
	(*d*) Actinomyces, or Actinomycetes	35
	(*e*) Algæ	37
	(*f*) Lichens	40
	(*g*) Viruses	42
III.	Short of Eternity	46
IV.	The Puzzle of the Heated Haystack	51
V.	Microbes come Alive	63
VI.	More about Growth	76
VII.	The Microbiology of Disinfectants	79
VIII.	Some Microbes come under Control	88
IX.	Using Your New Knowledge	96
X.	Some Other Experiments:	
	(*a*) In Liquid Media	104
	(*b*) On Solid Media	108
XI.	Counting the Millions	115
XII.	No-bacteriology	121
XIII.	Bad to be Good	125
XIV.	Microbes and the Home	133
XV.	What Microbes do in the Soil	141

CONTENTS

CHAPTER		PAGE
XVI.	BACTERIA AND THE GARDENER . . .	150
XVII.	MICROBIAL ASSOCIATIONS AND SUCCESSIONS .	159

A—G: SEVEN FOR SIXPENCE

A.	FAIRY RINGS	173
B.	LOOKING-GLASS CHEMISTRY . . .	180
C.	A CRUISE ON THE NORFOLK BROADS .	188
D.	THE MIRACULOUS BACILLUS . . .	197
E.	THE PRESENT STATE OF THE GREAT INTERNATIONAL FUNGUS-GUNNERY COMPETITION	203
F.	MICROBES AS SOURCES OF PERFUMES, DRUGS, DYES, AND FOOD	208
G.	STOMACH SARCINAE	215
	EPILOGUE	219
	GLOSSARY AND AMMUNITION . . .	234
	ENVOI, WITH ACKNOWLEDGMENTS . .	240
	INDEX	243

ILLUSTRATIONS

(*Between pages* 128–9)

HOW TO USE THIS BOOK

To the ordinary reader I would say: Just read it, and get what amusement you can out of it. The book has been designed to be very unlike a textbook.

But the book will probably be read by students and specialists in science, and since it contains a good deal of information that has never been published before, and some more information that is not yet well known, I have—largely for the selfish purpose of saving myself correspondence—put in references to scientific literature dealing with the more obscure points. Such specialists' references all occur as footnotes, and may be wholly neglected by the general reader.

When it is known to me, information about introductory works suitable for non-specialists has been collected at the ends of chapters.

Specialists in English may be assured that I have tried not to offer hostages to Mr. A. P. Herbert, M.P., though I have started—or perhaps averted (it is yet uncertain)—a new word war.

THE AUTHOR.

CHAPTER I

A SALTSPOONFUL OF SOIL

" I should see the garden far better," said Alice to herself, " if I could get to the top of that hill : and here's a path that leads straight to it—at least, no, it doesn't do that——" (. . .) " but I suppose it will at last. But how curiously it twists ! "

" Through the Looking-glass."

OURS is a busy, vexed, quarrelsome world. It contains about two thousand million people intent on gaining a living, on learning, on loving, on killing, on inventing, on changing, and on drifting. Every one is occupied with his or her ego. Conflicts arise, and stories of the more sensational conflicts are received in the newspaper offices, and are called news. The journalist pacing the pavement of Fleet Street knows that behind the façade of the big newspaper offices are telephones and teleprinters receiving news from the whole world.

There is no soil in Fleet Street, only pavement. Perhaps that is why the journalists miss the story of a conflict involving vastly greater numbers than the human population of the globe. If you were to pace the Downs instead of a dead pavement, your every footstep will cover a population many times greater than a couple of thousands of millions. There is at least that number of living things in a saltspoonful of soil. They teem, eat each other, and have their ups and downs in a perpetual conflict for food and existence. What a story ! Do you wonder that I am grateful to those pavement-bound journalists for leaving me an unparalleled scoop ? I shall not be able to exhaust its possibilities, or " cover every feature," as the journalists say, in one book, but I shall put you in a position to find out more for yourself about these microbes which live in myriads all around you.

MICROBES BY THE MILLION

Somewhere or other you may have seen an unlet advertising space filled after this fashion: " You are looking at this space. . . . Others will do so, if you put your advertisement here. For particulars apply . . ." Sometimes the challenge is put in the form of a query. " Why are you looking . . . ? " I may therefore suppose, as you have got so far with me, that you have a certain interest in following out Microbial News, or (to re-apply a phrase) News from the World of Neglected Dimensions.

The creatures dealt with in this book range in size from beings just visible to the naked eye, down to those that are about $\frac{1}{20,000}$th of an inch across and can only be seen with a powerful microscope. But though small, they are alive; they are involved in the struggle for existence, and they exist in perpetual conflict. That is what makes their study so interesting. Change—a new balance of power—a fresh dominance—news, in fact—is always being presented. But not by teleprinter; microbe news has to be recorded in other ways.

The method used for study of the modes of life of microbes belongs to the sciences collectively called microbiology. The name is perhaps not so very good; it suggests a small biology, whereas it really means the biology—the life-study, that is—of small things.

If at this stage you were to ask me to say what a microbe is, I could give no better definition to the term than by saying that a microbe is a form of life so small that a microscope has to be used to make it visible. Even so, some exceptions have to be apprehended. The egg of a mammal, such as a human being or a cat, is so small that it cannot be properly seen by what the German language calls the unweaponed eye; the male equivalent of such eggs is still smaller. Small, also, are pollen grains, which are the male " eggs " of plants. Such structures as these are not considered to be microbes, and possibly you will understand the reason without my stressing the point about an inde-

pendent existence. Microbes are very small Peter Pans: they never grow as *individuals* beyond a minute size.

This is the jumping-off point of the book. Like other writers on microbiology, I have laid stress on the fact of the smallness of micro-organisms. Unlike those other writers, however, I am not going to pre-occupy you with what can be seen under the probably unavailable microscope. Our present point of departure will be the fact that microbes live not only as individuals, but also in populations; given the chance, microbes form million populations even more easily than men and women drift into those agglomerations we call cities. It is, indeed, their enormous numbers that make microbes significant. The growth and decay of huge microbe populations produce effects of vital importance to ourselves.

The most sensational news often comes from conflicts set up in urban conditions of politics and business, and comes less frequently from the life-stories of isolated groups of people. The most instructive history is that which traces the rise and fall of cities, countries, and empires: the story of the growth of Chicago has more lessons for us than the biographies of a dozen Babbitts would have.

A population of micro-organisms—stimulated into being and growth in one spot by some especially favourable concatenation of conditions—is called a *colony*. Although a colony of bacteria, for example, may be so small that a microscope is needed to see it, I shall be on safe ground if for the purpose of this book we take the word *colony* to mean a microbial population which is large enough to be easily seen by the naked eye. I shall make use of the property of *colonial* growth (growth in *colonies*) to tell you how to study microbes by seeing them in the mass, *without a microscope at all.*

It is the point of departure which makes this book different from any other that has been written about bacteriology or about the other sciences that deal with microbes. I am

well aware that other writers on microbiology have stressed the importance of having a good (and consequently expensive) microscope in order to see microbes under the best possible conditions. The insistence on the need for a microscope for seeing microbes has naturally quenched the interest of those who would like to know something about this world of popularly neglected dimensions, but who have no time, room, or money to install a small laboratory. It would be easy for me to write a chapter or two to try to wheedle you into getting a microscope and some accessories; as Topsy did with her baby, I might maintain that it would not be much to make a fuss about—but I think that all but a few of you would remain unconvinced about the need for even a small beginning of an outfit for microscopy.

If I did convince you, it would be A Bad Thing. Though I don't want to labour the point, I may mention that this is probably the first book about microbes that has been printed in a first edition of 50,000. Now, if all the 50,000 of you (supposing I were *very* convincing) or any large number of you (to come down to more rational presumptions) sought forthwith to purchase microscopes and so forth, the swollen demand would lead to a sure rise in prices and to difficulties of supply. (I mention this chiefly to show you that scientific men are not invariably remote from everyday matters.)

I am taking the population-habit of microbes as our departure, before we set out on a voyage to new colonies, and experience the thrill of discovery. I shall occasionally have to refer back to microbes as seen under the microscope. I shall leave other microbiologists to hug the coast of sizes, and to discuss at length the size and shapes of what they suppose to be representative individual microbes; we shall deal with microbes in their teeming millions. It is, as I have said, by their vast numbers that microbes can influence our lives. For the moment, however, I am concerned

merely to take advantage of the fact that the aggregation of large numbers of microbes into a colony makes the mass visible to the naked eye, and hence simplifies their study without a microscope.

I have said that a microbe is a living thing that cannot be seen until it has been magnified considerably. Before setting off to found new vastly-populated colonies, let us see whether we can divide microbes into tiny plants and tiny animals and so forth. This sorting-out is conveniently done on the shore because it is not so easy as it seems. I note that you imagine that you have just decided that I have been guilty of loose writing. "And so forth. . . ! There are only *three* kingdoms of matter—the animal, the vegetable, and the mineral. Because it is alive, a microbe cannot be mineral!" No, it can't; but it is so very hard to say whether many microbes are animals or plants, that some knowledgeable people have suggested that a fourth kingdom should be created, intermediate between plants and animals. The name "protista" has been suggested for the members of the fourth kingdom. There is a good case, too, for the formation of a fifth kingdom, to include the viruses. These are bodies so very small that they have been called ultra-microbes, but nobody knows whether they are living or not. I shall ignore the viruses for the present, leaving consideration of them to a later chapter.

You will already have grasped the idea that the old division of matter into animal-vegetable-mineral is too simple. Like the belief in the indivisibility of the atom or the impossibility of the transmutation of elements, the division of things into three kingdoms was a satisfactory boxing of knowledge until knowledge overflowed. Scientific classification, then, is not immutable. It is merely a convenience. It is the packing and not the goods, except to a small number of scientific boxmakers. I shall, however, try to give a rough idea of the differences that have resulted in the establishment of animal and vegetable kingdoms, in

order to make the subdivisions of microbes more intelligible.

The main differences centre upon the modes of feeding. The modes of reproduction are very important indeed in establishing differences between different kinds (classes and species) of animals or plants. Thus they distinguish the kangaroos which are marsupials, from the reptiles which lay eggs, and the mammals which have a placenta and also bear their young alive. Broadly speaking, however, in the higher plants the methods employed for reproducing the species differ little, in principle, from the mode of reproduction in any higher animal. In the lowest plants and animals the methods of reproduction are practically identical. Digestion, and not sex, forms the primary basis of classification as animal or plant. In simpler words, to tell whether an organism, that is, a living thing, is a plant, or an animal, we need the answer to Alice's question at the Mad Tea-Party: "What do they live on?" Alice took a special interest in questions of eating and drinking.

You will remember the Rocking-Horse Fly which was made entirely of wood, and got about by swinging itself from branch to branch. "'What does it live on?' Alice asked with great curiosity. 'Sap and sawdust,' said the Gnat.'"

My point is that the Rocking-Horse Fly had to have its ingredients ready-made for it. Sap and sawdust are plant products and are complex, but they are built up by the plant from very simple chemical compounds, such as carbon dioxide, water, and nitrates. Therefore, in saying that animals are destructive in their feeding, and that plants are constructive, we have broached a fundamental truth.

A great deal follows from this. We seem to have wandered very far indeed from the microbes and their struggle, but believe me, you are very near to understanding much more than the differences between plants and animals. You are very close to understanding what microbes do in

the earth, and those activities are at the bottom of our existence on this planet.

Most animals (except such very humble specks of living matter as amœbæ) have something corresponding to a mouth. They use their mouths in much the same way as we use ours—to take in organized food, by which we mean a foodstuff that has a complex structure, because it is or has been part, or even the whole, of another living thing.

No plant has a mouth. The insect-catching plants are a special case, but need not detain us here. Generally speaking, plants feed on the simplest substances, which they build up into highly-elaborated stuffs.

You are now in possession of enough information to see that there is a tendency towards a food cycle, or, a circular process of feeding. From simple matter, plants build up complex plant-tissue. Some animals feed on plants; some animals feed on plant-eating animals.

Thus, simple stuffs are built up into big organisms via plants. But the big organisms—animals and plants—are not immortal. They die, and then what of the stores of material they represent? Obviously, our cycle is not complete. You can readily see that if the stuffs built up into big living things were not returned to circulation, all life must come to an end, partly for lack of fresh material, and partly because the ground would be cumbered with dead things. The higher plants cannot make use of one another's tissues or of the bodies of animals, while these are intact.

The cycle is completed by the microbes, which are essentially preparers of plant-food. Their work is known by unpleasant names—decay, decomposition, and so forth. Nevertheless, you are now able to appreciate how essential a process decay is. Decay or decomposition is looked upon as something foul, because it is often accompanied by unpleasant smells. Take de-composition in its literal sense, which happens to be a true sense, and it is merely the oppo-

site of re-composition, which is surely nothing to be frightened about.

The inhabitants of that world of neglected dimensions, the microbes, are like the house-breakers in a city which is for ever building anew out of the materials of its old outgrown or out-moded dwellings. Human house-breakers do not alter their materials, but microbes refashion theirs, and infuse them with the mysteriousness of life once more.

INTERLUDE

Tune: *The Keel Row*

They all go to Adco, to Adco, to Adco;
They all go to Adco, to Adco in the end.
Old hats and boots and braces,
My lady's silks and laces——
Just turn 'em into Adco, and dump 'em on the land.

We all go to Adco . . . to Adco in the end.
(*lento*) And when our time is ended
And life in us suspended:
We'll all go to Adco, to Adco in the end.
(Pardonable exaggeration in a song at a Rothamsted Christmas Party Pantomime.)

ADCO is the name given indifferently to a compost ("artificial farmyard manure") of straw, garden waste, etc., prepared with the aid of the patented "Adco" powder, and to the powder itself. The former is meant in the above verses. The "Adco" powder is nothing more than a mixture of chemicals, chiefly nitrogenous; when this powder is added in prescribed quantities to a moistened heap of decomposable material, such as straw, and garden wastes, the rotting proceeds under optimal conditions, and the final product is substantially identical with good farmyard manure. No addition of bacteria, or other microbial inoculation, is necessary, as the microbes already present on the vegetable material are induced by the moisture and the food substances in that material, *plus* those added in the powder, to proliferate and grow in the correct manner for the formation of a useful plant food. In doing so, the microbes undergo a complicated series of changes in kinds and number, various groups predominating in succession. The conflict is, in fact, controlled by chemicals.

The marketing of "Adco" is one result of an extended study of modes of decomposition of vegetable materials

undertaken at Rothamsted, which has led to the fundamental concept that for the optimal and agriculturally most economical rotting-down of vegetable wastes, the ratio of carbon to nitrogen must be maintained at about 10 to 1. If this is done—as it is in rationally-made farmyard manure and in the Adco compost—the successive conflicts of the microbes responsible for the decompositions resolve themselves into an outcome most beneficial to man. It has also led to some reference to Adco becoming almost inevitable on the stage at the Christmas Party.

A few years ago a small group of women connected with rural industries or some such non-scientific activities, visited Rothamsted. Playing about in the Bacteriological Laboratory there happened to be a kitten in transit from the home of one colleague to another's. On being shown into the lab., the first thing the leader of this group saw was the kitten; hereupon, ignoring ceremony, she exclaimed impetuously and apparently without a trace of irony: " So you *are* human after all ! "

I think the very nicest thing about Rothamsted is a detail of the Christmas Party, which consists of a stage show followed by a dance. It is, of course, held in the evening. Though most, if not all, of the graduate staff and old colleagues and students possess a dinner-jacket suit, the rule is strictly morning dress. This rule is observed by the graduates and their wives, for it exists in order that such as the ploughmen, and the little girl who has just left the elementary school to join our assistant staff, shall not be outshone. Perhaps now you have enough data to form a conclusion as to whether scientists are human after all.

CHAPTER II

WHAT MICROBES ARE

" Consider your verdict," the King said to the jury.
" Not yet, not yet ! " the Rabbit hastily said. " There's a great deal to come before that ! "

" Alice in Wonderland."

(*a*) FUNGI

(Yeasts, moulds, mushrooms, etc.)

THERE are highly-organized and quite large plants which lack some essential part popularly regarded as characteristic of a plant. Thus, the sandal-tree of Mysore—an indubitable tree growing out of the ground, and of which the wood furnishes the Mysore sandal-wood oil of perfumery commerce—has no true roots of its own for absorbing nutriment from the soil, and for that purpose is parasitic on roots of other trees. Broom-rape is, and the dodder plant was, a fairly common plant of arable land in England; these plants have flowers, and can propagate themselves by means of seed, but have no chlorophyll. They have no green colour.

The green colour of leaves and stems of ordinary plants is due to chlorophyll, an intensely blue-green material that enables plants to make direct use of light for the production of sugars and starches from carbon dioxide and water. Chlorophyll belongs to a class of substances known to chemists as *catalysts*. Catalysts have the property of promoting a chemical reaction between two substances without themselves suffering obvious change in the process. Chlorophyll, for example, promotes the union of carbon dioxide and water in the presence of suitable light, and sugar is formed, which the plant further builds up into starches and other substances. Most green

parts of plants contain about 3 per cent. of sugar, and starch in addition.

The union of carbon dioxide and water to form more than traces of sugar has never been performed in any laboratory, whatever catalyst was used. The alchemy of the green plant brings about the combination of carbon dioxide and water to sugar readily enough under the influence of light and chlorophyll; the presence of chlorophyll is essential for this combination to occur, though the chlorophyll suffers no loss and undergoes no apparent change during its performance. Mr. E. C. Large's fascinating novel *Sugar in the Air* centres round the artificial production of sugar with the aid of an imaginary " blue catalyst." Since Mr. Large is chemist enough to give scientific explanations that are accurate—granted his fanciful premise—it is clear that it was chlorophyll that he had in mind when he " invented " his " blue catalyst " for producing sugar from the air.

Two classes of plants have avoided the need for using any chlorophyll of their own. One of these classes is typified by the broom-rape and the dodder: they are parasitic on green (chlorophyll-bearing) plants, and obtain their sugars by " vampiring " their host, but in other respects they substantially resemble ordinary plants. Their leaves may be only vestigial—since leaves are the main seat of sugar-production in plants, the broom-rape and the dodder have no need of leaves—but the flowers are normal; the seeds are profusely produced, are like ordinary seeds, and germinate in the ordinary way.

The fungi form the other class of plants that have no chlorophyll. There is no satisfactory definition of fungi. They may be regarded as plants low in the evolutionary scale, devoid of chlorophyll, and able to live without light. Some fungi are parasitic, but many are not. Those that are parasitic get their nutriment from the host (though some fungi also have a non-parasitic stage in their life); the host

may be animal or vegetable, but I think that practically no one species of fungus is capable of having both an animal and a vegetable host.

The fungi that are not parasitic must find their sugar ready-formed. For non-parasitic organisms, the commonest source of sugar in nature is the remains of recently-dead plants. I say *dead*, because if the organisms lived on live plants and animals, they would be parasitic. Hence the great importance and activity of fungi in performing the earliest stages of breakdown and decay of vegetable residues. The microbial habit of non-parasitic feeding on dead matter is called *saprophytism*. Most parasitic fungi are pathogenic (disease-producing), while most of the more pleasingly helpful fungi are saprophytic.

Some saprophytic fungi, however, are not very welcome to us. Thus, consider the bluish mould that often occurs on an orange, or the apparently more greenish one common on a lemon. From the commercial point of view, such mould-growths constitute something very like a " disease," and reduce the marketable value of the fruit. Whenever you see such a mould growth on a fruit, you may assume that the fruit has been damaged first. The damage may have been nothing more than a slight blow at the spot colonized by the mould, but the blow has destroyed the integrity and vitality of the fruit *at that spot*, and some one or other of the ever-present *Penicillium* species (blue-green moulds) has found conditions to suit it, so it grows. The *Penicillium* group of moulds are suited by the conditions offered by damaged citrus fruits, hence the frequency with which they appear on market specimens of these fruits. *Penicillia*, growing on such sites, are doing no more than expressing the urge to restore the locally-damaged, and devitalized, if not dead, plant-material into circulation as food for higher plants once more.

The fundamental mode of propagation and reproduction of fungi is by mycelium—the microscopically-thin threads

which you can often see in masses running through a mushroom-bed or an over-dry compost heap. (See the diagrams on pages 36, 176, and 178.) All fungi can ideally reproduce by means of mycelium. The yeasts (and some other fungi) do not have true mycelium, but are considered to have come *down* in the evolutionary scale from forms that did have it. The mycelium is simply a fine thread of fungus tissue, and is the essential part of the fungus. Mushrooms and toadstools are only the fruiting-bodies or spore-bearers of fungi of which the mycelium is ramifying in the soil or wood on which the mushroom " fungi " are growing. Mycelium grows; an older part of the mycelium thread can be decaying even while another part is growing; hence it is often difficult to say where the fungus begins and ends, or to say which is the individual. Any little bit of live mycelium can grow and reproduce its kind, complete with fruiting bodies or other complicated structures, if it finds the right conditions.

In saying " any little bit," I mean any piece large enough to contain one cell, with nucleus. The cell is the unit of biological matter; the nucleus is the essential vital kernel of the cell. Cells are usually of microscopic size, and are always of microscopic size in the fungi—however large and visible the fungal structure may be. A mycelium is a string of cells; the string may be many yards (many thousands of cells) long, but essentially its diameter is that of only one cell. The yeasts consist of one-celled individuals; most other fungi are multi-cellular at some stage of their life.

Multi-cellular fungi can develop a single-celled form known as spore. Bacteria are all unicellular, and some bacteria forms unicellular structures which are also called " spores." Bacterial and fungal " spores " are rather different. You will learn later what a bacterial spore is; for the present I will confine the discussion to fungal spores.

The spore of a fungus is a special form of mycelium, and often contains only one nucleus. It is a microscopic struc-

ture, something like the germ of a seed, for it contains in itself (just as any other mycelial cell does) the potentialities of reproducing a plant like its parent. Spores may be formed asexually or after a sexual process. Since the ordinary mycelium is capable of reproducing itself, you may ask: Why do fungi produce spores? The answer is that spores are produced for one of two purposes: for dispersal, or for resisting unkindly external conditions.

The " dispersal " spore is formed for much the same purpose as a dandelion seed is. The " resting " type of spore has no exact analogue in ordinary botany. It is usually produced when the ordinary (so-called " vegetative ") form of the fungus is threatened by drought or by some other external factor tending to reduce availability of the food supply—or, of course, by approaching exhaustion of the food supply by growth of the fungus itself. Not all fungi produce both types of spore; the ordinary mushroom, for example, has only the " dispersal " type. Millions of spores of mushrooms supply the pinkish-brown colour to the underside of the " cap "; similarly with toadstools. Although such spores cannot be individually seen by the naked eye, several ingenious methods have been devised for seeing them " in bulk " after they have been released in millions by the fungus.

Spores of either type occur collected into enveloped masses of many forms. These are known as " sporangia " and by other terms—to the bewilderment of the beginner in mycology (the study of fungi, also called *fungology*). Such packed masses are sometimes large enough to be picked out from other material (such as soil) by use of the unaided eye and fingers. The projectiles of fungus-guns (see p. 203) are filled spore-cases designed for a peculiar method of dispersal.

The little clumps of fungi such as green *Penicillium* on an orange, and grey *Mucor* on damp bread, are known as " colonies." The fungus settles down as some microscopic

speck of mycelium, or as a spore; then, because it finds the " land " vacant and suitable, it grows and occupies as much space as it can. It starts by sending out mycelium into the devitalized tissue; when the most handy food supply begins to come to an end, the fungus sends up tiny aerial stems which ultimately bear spores, to be dispersed all over the place. Such spores will not normally grow on still healthy [1] parts of the orange skin or on dry parts of the bread; but, while the spores are developing, or even before that, the mycelium inside the food material (*substrate*) will be seeking peaceful penetration of the still intact parts, if their condition is suitable for its growth. The fungal colony develops from one individual spore or piece, but is composed of several parts (ramifying mycelium, spore-bearing stalks, spores); and it is difficult to say whether it is one individual or many.

The number of fungi in resting soil (*i.e.* not recently manured with decomposable matter) is about one million per gram, but, as already hinted at, it is difficult to say what a fungal individual is. The figure just given should be taken to mean " one million spores, or small pieces of mycelium, capable of development," but obviously a piece of mycelium may, if shaken with soil, or otherwise disintegrated, give rise to more than one organism.

Fungi are so diverse that they almost baffle generalizations beyond those already given. Later in the book are given some methods for the growing of yeasts, which easily form colonies on artificial media. It is easy to grow some possibly more " typical " fungi, such as the *Penicillia*, but experiments of that sort may not be desirable in the home, where, presumably, they are already sufficiently familiar. Fungi of the *Penicillium* types are sure to turn up on nutrient " plates " made and inoculated according to the directions given in Chapter X (b). Further than that we cannot, I think, go at present.

[1] See note on p. 61.

(*b*) PROTOZOA;
and something about measurements

The one-celled animals are known as Protozoa. The causal organism of malaria is an example of a prozotoön. It can find in a single human blood corpuscle, of which there are over thirty thousand million in a cubic inch of normal blood, a comfortable niche for its equivalent of nesting and setting up house and home. The malarial organisms are all equipped with a complicated nucleus, and have recognizable animal characteristics.

We must meet the difficulty of units of measurements. I have just got over one stile by referring the sizes of a minute animal to another minute but relatively well-known structure, the red blood cell. However, even in Great Britain, medical men do not reckon the number of red blood cells as so many per ounce or cubic inch of blood: they invariably use the metric system for this, and speak of the number of red cells per cubic millimetre. A millimetre is about a twenty-fifth of an inch; it is as long as the stroke inside this "e." You may imagine a cubic millimetre as a little cubical box as long, as wide, and as high, as the stroke of this letter "e". A cubic millimetre of ordinary blood contains $4\frac{1}{2}$ to $5\frac{1}{2}$ million red cells. Roughly speaking, most microbes are of the same size as a red blood cell: bacteria are usually small enough for a number of them to lie on a red cell, yeasts are not much larger than a red cell, and some protozoa and microscopic plants are several times larger than a red cell. This will give a rough idea of the relative size of the organism usually understood as microbes. But since several millions of red cells, not at all tightly packed, can float in the fluid of a cubic millimetre of blood, the millimetre is an inconveniently large measure of microbial size. To measure the size of a microbe in millimetres is about as awkward as it would be to give the dimensions of a doll's house in miles. It is

possible, but hardly practicable, and would mean too frequent use of noughts after the decimal point.

Microbiologists have completed the nomenclature of the metric system to the extent of devising the word " micron " for the thousandth of a millimetre. Apparently the original propounders of the metric system did not foresee the need for divisions smaller than a thousandth of any unit. The Greek letter μ (usually pronounced *mew*) is often used as a symbol for the *micron*, the plural of which seems to be preferably *microns*. The micron (μ) is a suitable " unit " with which to measure microbes. You will recall that a millimetre is as long as the stroke of the small " e " in this text; and a square millimetre is this size ▪ . On a square millimetre, a million small bacteria measuring about one micron in diameter—say, the cocci—could be laid without much overlapping in a single layer of a thousand rows having a thousand in each row. There are 1,000,000,000 (a thousand million) cubic microns in a cubic millimetre. If bacteria about a micron in diameter were packed into a cubic millimetre, about 1,000,000,000 of them would be got in; there would be a thousand layers each containing a million bacteria. This total is only about half the number that is normally present in a saltspoonful of soil, but the 2,000,000,000 population in a saltspoonful of soil occupies very little space after all. In fact, twice that number will occupy less than a half of 1 per cent. of the volume of the soil.

The normal red blood cell is about 25μ in diameter. The ordinary protozoa of soil or of pond water range about 5μ to 50μ in diameter or length; some are considerably larger, and, like the green *Stentor* of ponds, or the luminous *Noctiluca* which is responsible for much of the phosphorescence seen at sea, are individually just visible to the naked eye. Protozoa as a class are highly various in shape. Most of the soil protozoa are complicated in structure, while others, such as the parasitic protozoa

causing tropical diseases, undergo very complicated life-cycles.

A type of protozoa often taken by the textbook for introductory study is the amœba (plural, amœbæ). This has no shape of its own. It is usually described as a speck of living jelly, and as crawling about in a peculiarly amorphous manner: extending any part of its body to enwrap and engulf bits of food. Presumably it is spherical when in the resting condition in a liquid, but it is usually studied on a glass " slide " under a microscope, so that it appears flattened.

There are two other groups of free-living protozoa, distinguished from the amœbæ and from each other by their modes of movement: these are the flagellates, and the ciliates. The majority of protozoa can form cysts, which are resistant forms analogous to bacterial spores. Protozoa occur in the brine-lakes in deep salt mines, having been brought in from outside and having become tolerant of the high concentration of salt.

Many of the larger protozoa appear green, because, as seen, they are not merely animals. Each such individual protozoön includes a number of individuals of one species of algæ (microscopic green plants) somehow in its make-up. Such associated algæ (generically called *Zoöchlorellæ*) are capable of leading an independent existence, but the protozoa which shelter them are believed not to be able to live continuously without the algæ, which probably provide sugars for their animal host. It has been said that such protozoa are plants by day and animals by night!

The numbers of protozoa in soil seldom attain a million per gram. Protozoa do not form colonies, and there is no simple way of studying any aspect of their behaviour (except luminescence in the luminous protozoa) without the aid of a microscope. Hence, they are mentioned not more than occasionally in this book.

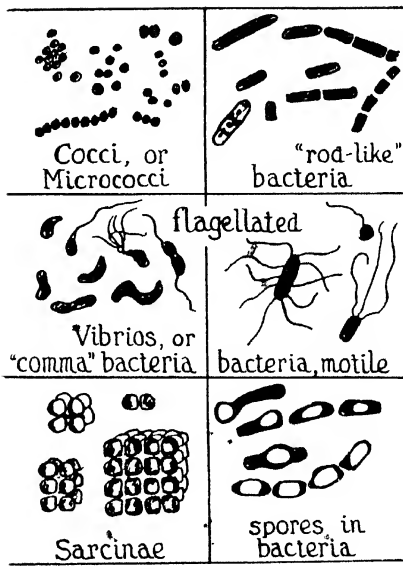


Fig. 1.—Shapes and features of some typical bacteria, all drawn to the same scale. As bacteria are usually almost transparent, it is usually necessary to stain them for microscopic examination. The bacteria seen here may be assumed to have been stained (compare Fig. 3 for appearance of unstained bacteria) and to appear rather larger than they would seem if seen in the usual bacteriological microscope. Special methods of staining are necessary to make flagella visible, as the ordinary methods do not give any indication of their presence. Spores fail to stain by ordinary methods, and are represented by the white shapes in the lowest right-hand drawing. Spores usually appear especially bright because of their acting like little lenses towards the light of the microscope lamp. Though developing inside the bacterial cells, as shown in the drawing, spores often exist free, *i.e.* without any part of the cell-wall from which they have been released.

(*c*) Bacteria

As already explained, a coccus (also called a micrococcus) is commonly about 1μ in diameter, and is roughly spherical. It is the smallest type of bacteria. Many kinds of bacteria are rod-shaped, and the rods are 1μ more or less in diameter, and may be from 2μ to 10μ long. There are numerous textbook divisions of bacteria into classes depending upon shape and modes of arrangement, but most of these divisions are based on the older teachings of medical bacteriology, which assumed that the behaviour of bacteria was so far invariable that they could be grouped into species, as plants are, according to definite descriptions. The medical bacteriologists were in the main right so far as their special bacteria—accustomed to the constant conditions of the human body—were concerned. In the newer branches of bacteriology less reliance is placed upon constancy of bacterial characteristics; hence it seems unnecessary to indulge here in hackneyed explanations of technical terms which I seldom use myself.

Some kinds of rod-like bacteria can develop spores for the purpose of resisting adverse conditions, but there is never more than one spore to a cell. The term " bacillus " was at one time reserved for spore-forming bacteria; I mention this merely because you will probably have met the word, and may be expecting to know what the difference is between a bacillus and a bacterium. Practically, there isn't any now, the word " bacillus " being almost obsolete.

Some kinds of bacteria develop flagella (singular, *flagellum*) by means of which they can swim (become motile). Without flagella, bacteria can move only passively, as in a current.

A few typical forms of bacteria are shown in Fig. 1. Fig. 2 shows the life-cycle of a single kind of bacterium—the non-spore-forming bacterium responsible for the development of nodules on leguminous plants. As this

Fig. 2[1].—Forms exhibited successively by the text-book type of nodule bacteria; *i.e.* the normal beneficial type which fixes appreciable amounts of atmospheric nitrogen when in the nodules of leguminous plants. These bacteria assume similar forms in the soil, and to a less marked extent in laboratory culture media. You will note the transition from flagellated to ordinary form, from coccus to rod and back to coccus.

The newly-discovered non-beneficial forms of nodule bacteria, which form nodules but do not benefit their host-plant appreciably by fixing nitrogen, undergo still another succession of changes. The non-beneficial forms of clover nodule bacteria are prevalent in soils of the Welsh uplands; the most outstanding example has been that found on the estate of Mr. S. M. Bligh, near Builth Wells in Breconshire. Such abnormal bacteria show, instead of the rod form, a form which is nearly spherical and is new to science: we have called it the "globoid."

[1] After H. G. Thornton and N. Gangulee, *Proc. Roy. Soc. Lond.*, 1926, **99**, 427 [16900].

bacterium undergoes a similar life-cycle both in the soil and in the nodule, it cannot be said to have one special form. It is nearly as true of bacteria, as it is of bees, that " they never do anything invariably."

Names of bacterial " species " are to-day used with the tongue in the cheek, that is to say, as a possibly convenient handle, but without greater claim for their validity. Good bacteriologists are often content to speak of " a short rod," and to give their bacterium a number or initials. " Y.B." —*i.e.* " yellow bacterium "—is a species or strain which two of my colleagues have kept alive and under observation for years, scorning to give it a pseudo-scientific name.

The ways in which bacteria can aggregate are of interest in relation to discussions later in the book. Cocci and rods can severally form chains, either without any obvious linkage-mechanism, or with a loose investment of gum or mucilage, or with a definite sheath. The nodule bacteria are normally free, but at the moment of invading a plant a number of the bacterial cells agglutinate into a gummy streak called an infection thread; similar threads occur inside the formed nodule when the bacteria are passing into a previously sterile cell of the nodule. Somewhat similar threads are formed in the " ginger-beer plant " and other associations of bacteria with a yeast (see p. 163). The large coccoid, alga-like, bacteria of the *Cladothrix* type are normally ensheathed in a definite envelope, which manifests " false branching " (*i.e.* branching of the sheath, and not of the bacteria) as is shown in Fig. 4. It is unfortunate that the term " filamentous form " should be used, unless it is stated which of the several possible types of filament is meant.

Some long rod-like bacteria can be seen to divide by forming a septum—a sort of wall across the cell, about midway between the ends—not lengthwise. A constriction appears all round the cell where the septum joins the

enveloping wall of the cell. This constriction becomes more and more waisted until the original single cell breaks into two, the septum helping to make the new ends of the two cells now formed. The picture will help. The septum probably shrinks down so as to occupy only the centres of the two new ends, the rest of the new hemispherical ends being formed, either from newly-grown cell-wall, or else from the material of the old side walls. The rod-like bacteria are too small for it to be seen what actually happens, and the difficulty of seeing what happens

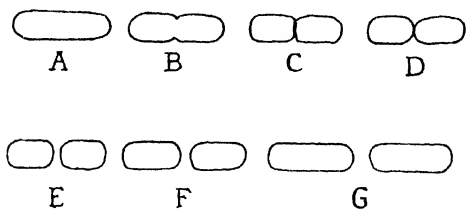


Fig. 3.—The usual mode of increase of bacteria by division in the middle and subsequent growth in size of the two "halves" until the length of the "parent" is reached by both of the resulting cells. Greatly enlarged.

during division is still more pronounced in other bacteria: the cocci, vibrios, etc.

There is apparently little or no growth in length or size of bacteria during division. Each of the bacteria resulting from the transverse fission that has just been described have a trifle more than half the length of the original. It is usually after division that the bacteria grow in size, until they have reached a size similar to that of the original. They are both then able to divide again, and so indefinitely with them and with their progeny.

The above-given mode of division is the one which

superficially appears simplest, though it is very hard to understand anything whatever about it. It is the mode of bacterial increase usually described in the textbooks of bacteriology. There are a number of other ways of bacterial multiplication, all taking place by division. Some bacteria—especially the nearly spherical sarcinae—divide apparently into very fairly equal quarters, or at least into two followed by two on another side, in a way which is rather difficult to express neatly, except by saying that the result of the division tends to look like a substantially rectangular or cubical packet of slightly flattened balls. A picture is a great help (see Fig. 1). The " packet " appearance is almost characteristic of the sarcinae.

The modes of division mentioned above as adopted by bacteria are asexual. That is, there is no distinction into male and female, and the conjunction of sexes therefore does not come into the question. A sexual process has been suggested to be a mode of reproduction for a few kinds of bacteria, but the evidence for bacterial sexuality is not altogether clear.

(*d*) Actinomyces, or Actinomycetes

These are a class not easy to define, but intermediate between the bacteria and the fungi. They are like fungal mycelium, or very long bacteria, have no obvious nucleus, and can form spores something like fungal spores. The mycelium is commonly branched, and may give rise to spores at the tip (S in Fig. 4).

Many actinomycetes have a pronounced earthy odour. Actinomycetes probably have a share with the lower true fungi in producing the musty odour of cellars. The actinomycetes have been held to be chiefly responsible for the odour of soil. As this odour is not always perceptible, but is most marked after a shower on a hot day, I have elsewhere suggested that the odour is largely due to an

effect of moisture on actinomycete spores, which would tend to be produced during a spell of dry weather.

Soil actinomycetes form small variously-coloured colo-

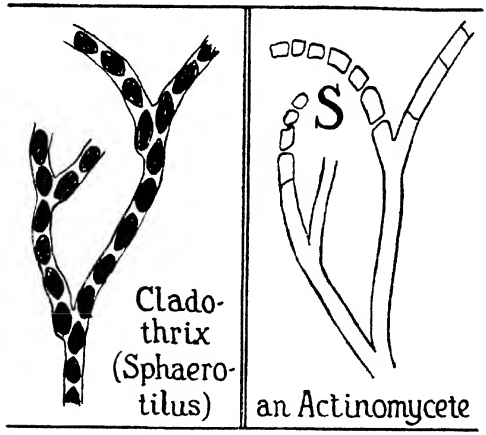


Fig. 4.—Contrast between false branching (left) and true branching (right). *Cladothrix* is a genus of large coccoid bacteria which normally grow in rows surrounded by a continuous sheath; it is the sheath which branches. In actinomycetes, the organism itself grows as a mycelium which shows branching in the ordinary sense. The mycelium of actinomycetes is almost indistinguishable from the mycelium of many fungi, hence this drawing of the appearance of actinomycetes will serve to give an idea of the appearance of fungus mycelium. These are on about the same scale as Fig. 1. Whereas actinomycetes are intermediate between the bacteria and the fungi, *Cladothrix* is possibly intermediate between the bacteria and the algæ. Many microscopic algæ grow in chains much as *Cladothrix* does.

nies resembling compact dense *Penicillia*, and will probably be met with by anyone trying to grow either bacteria or fungi from soil. They should not be sniffed too hard. A better trick is to smell the *cover* of the culture-plate !

(*e*) ALGÆ

An alga is essentially a chlorophyll-bearing plant without true roots, and without distinction of stem and leaf. It may be microscopic, as are the diatoms and the soil algæ, or it may be several feet or yards long, and easily visible, as the seaweeds are: they are algæ. The inclusion of such apparently diverse organisms under one class-name is an example of the beautiful but sometimes unsatisfying neatness of botanical classification.

In the Pelican *Outline of the Universe* (Vol. 2, pp. 256 and 257), Mr. J. G. Crowther has pointed out that the ordinary associations of the word " root " are linkages with the idea of fundamentality, whereas the essential activity of a plant is (he says) in the top, not in the roots. Certainly the evolutionarily earlier plants have no roots, and appear to manage quite well without them. Some of the seaweeds have false roots which serve only as anchorages, and, unlike the roots of the higher land-plants, take no part in the absorption of nutrients.

Algæ all resemble the higher plants in being provided with chlorophyll for trapping the energy of sunlight to good purpose. Some of the seaweeds, as you know, are distinctly brown or even red. In the soil there are green, blue-green, and brown algæ. Whether large or microscopic, the algæ which are not visibly green have as much chlorophyll in proportion to their size as have the green algæ or other green plants, but in the red and brown algæ the green colour is obscured by the presence of red pigments.

You will see that seaweeds, diatoms, and other algæ of soil and water are alike in not needing roots for the uptake of nutrient substances. Unlike the rooted plants, the algæ are wholly bathed in nutrient solutions. This is clear when the seaweeds and freshwater algæ are considered, but is not so clear that the ordinary soil algæ live in an abundance of water. The water which bathes them is the film which

covers the soil particles and serves as the nutrient solution for all the other soil microbes. The water film is always present, for even dry soil, so dry as to be dusty in the hand, contains at least 6 or 7 per cent. by weight of water, always provided that the soil has been merely air-dried and not artificially dried, or has not been taken from the surface of a sun-baked area. The soil algæ, being of microscopic size, are able to live in the films of soil moisture. The green algæ that can often be seen on walls occur only when the wall is actually damp. " A rolling stone gathers no moss " —*pierre qui roule n'amasse pas mousse*—is it not more often algæ which colonize a sedentary stone ?

A puzzle less easy to solve is what algæ, as chlorophyll-bearing plants, are doing in the soil where they cannot get any sunlight unless they happen to arrive on the surface. Perhaps that is the chance they live for ! It may be assumed that the soil algæ, though equipped with chlorophyll to take advantage of the energy of sunlight for building up carbohydrates, can also live without sunlight, but it is not known how they carry out their vital processes in the depth of the soil. Possibly they then live in a state of suspended activity, or they may live saprophytically, as the bacteria do, and compete with the bacteria and other microbes for decomposable food-material. Some microscopic soil algæ have been shown to share with a few specialized kinds of bacteria the property of fixing atmospheric nitrogen and converting that otherwise biologically-inert gas into compounds which higher plants can profitably use. This is especially important in tropical rice-fields.[1]

There is no satisfactory method of counting algæ in soil. An expert opinion is that the number is about 100,000 per gram. On this basis the algæ form only about one-third as much of the soil's living matter as do the protozoa, and only about one-twentieth as much as the bacteria.

The microscopic algæ (and many algæ which are not

[1] P. K. De, *Proc. Roy. Soc. Lond., B*, 1939, **127**, 121 [16900].

microscopic !) all multiply by division, but their division is not as apparently simple as that of the bacteria. In the sexual modes of reproduction, division occurs only after fusion of the nucleus of a male cell with the nucleus of a female cell. In most asexual modes of reproduction, division occurs apparently spontaneously. Little is known about a bacterial nucleus. In the microscopic algæ, which are true plants, and in other microbes (except bacteria), the nucleus divides first—also apparently spontaneously; and when the cell is sufficiently uncomfortable owing to the inclusion within it of two nuclei, the cell divides into equal halves. The nucleus also divides into equal halves, and each half of the old cell takes half the nucleus with it. This is how algæ divide.

Human beings and other mammals start life in a similar manner as a single cell, but the mammalian single-celled embryo is formed by the fusion of a male sperm-cell with a female egg. It then has one nucleus. When this nucleus and the cell which contains it divide into two, the halves are nearly but not quite identical. The difference that exists is implemented in later embryonic life by manifestations of a definite sex in the embryo, as well as by differentiation into head, limbs, etc. The algal nucleus, together with its cell, divides neatly into two, in such a manner that one half is, so far as we can tell, identically similar to the other half, and also identically similar to the original alga before division.

Algæ have some commercial importance. One of the chief commercial products of algæ as such is agar-agar, which is the purified and dried sap of several species of seaweeds growing near the Chinese, Eastern Russian, and Japanese coasts. Agar (for short) is of great utility in bacteriological culture-media. From the ashes of seaweeds, iodine and soda are extracted on a small scale. There are other commercial applications of large algæ. Microscopic algæ have no large-scale use when recent, but there are vast deposits of fossil siliceous skeletons of diatoms; under the

names Kieselguhr and Tripoli powder these minerals have a large number of applications. Kieselguhr is the absorbent which, when mixed with nitro-glycerine, a liquid, forms the tractable paste known as dynamite. Tripoli is an ingredient of many polishing powders. Under the microscope, broken skeletons of several species of diatoms can often be seen in such powders. Choice specimens of single diatoms, or rather their recent skeletons—the living matter having first been destroyed—are sold for as much as half-a-crown each for the delectation of microscope hobbyists. The fine structures of the diatom skeletons form exceedingly minute regular patterns; determination of the extent to which the details of these patterns can be seen and distinguished, forms a severe test for the quality and adjustment of even the best microscope.

On p. 84 a method is given to enable you to grow some algæ, provided that you are not particular about which algæ you grow.

(*f*) Lichens

Pronounced " ly-ken (s)." From the point of view of the teacher of biology, lichens provide a peculiarly interesting peg upon which to hang a disquisition on " What is a species ? " A lichen is a plant. You can see lichens as drab scaly growths on tree-trunks and old walls, and they furnish a good deal of local colour to novelists who wish to attach the idea of age to a building. Lichens are frequently confused with mosses, and amongst French-speaking people the word *mousse* is commonly employed for both. " Iceland moss " is a lichen. Lichens and mosses are small plants, but mosses, not being truly of microscopic size, are outside the scope of this work. I include lichens in this outline because they are partnerships between a microscopic fungus and a microscopic alga; the partnership results in a plant big enough to be seen by the naked eye.

The curious thing about each species of lichen is that it is a close partnership between a fungus and an alga, both capable of separate existence, the algæ (at least) being recognizable as well-defined algal species. It is as if one were to add F to A and call the result L. L is in fact much more than F and A; it is a sort of living monogram, in which F and A are interdependent, so that the result is recognizable as an entity. Lichens are recognizable by experts as species from their external characteristics alone, without necessarily appealing to the species of alga and fungus which together compose each lichen. The lichen species are in that respect comparable to those protozoa which are a partnership of a protozoön proper with algæ. The fungal partners in lichens differ in some respects from free-living fungi. The algal partners can and do lead independent existences in nature. In a lichen the fungus is the predominant partner: the reproductive body (spore) of a lichen usually contains only its fungus. After germination of this spore, the fungus grows out and seeks its algal consort; if none of the suitable kind happens to be near, the fungus subsists alone for a time, and then perishes. When a suitable algal species is at hand, threads of fungus first twine around the algal organisms, then develop special structures which give rigidity to the new lichen, and also express its characteristic pattern as a species.

Laboratory attempts to produce lichens by growing a lichen spore (fungal) with a pure culture of an appropriate alga have not been very successful: something more than the mere presence of both partners seems to be required in most cases, and artificial media able to supply this something have not, I think, yet been devised.

Lichens are not parasites. Even on trees they live independently of the support. They are good colonizers of bare territory ill-adapted to other vegetation. They visibly require very little but moisture, and can live on rocks and tree trunks, where food of ordinary plants is at a mini-

mum. Though resistant to drying, lichens prefer moist situations; their frequent occurrence on one side (the moist side) of a tree is an illustration of this. Though the food requirements of lichens are small, they are not negligible, and the slow disintegration by lichens of rocks and other almost barren surfaces leads, after detachment and decay of the lichens, to the formation of soil.

A few years ago a philosophical distinction was conferred on the lichens by the discovery by an Irish chemist in an Irish lichen of a carbon compound containing chlorine. It is very remarkable, in view of the abundance of chlorine in the sea, in rock-salt, and elsewhere, and in view of the ease and certainty with which carbon compounds containing chlorine have for many years been prepared in factories, that until 1934 no natural organic compound of chlorine was known: a lichen yielded the first.

(*g*) Viruses

These ought to be mentioned, though they are not microbes, but sub-microbes (or ultra-microbes). They may be defined as particulate bodies capable of causing infectious disease in animals or plants, and too small to be visible with even the best microscope. Note (1): All bodies are particulate, of course; hence, for " bodies," substitute " organisms " or " substances " as you prefer, after reading the rest of this section. Note (2): This definition will not be changed by improvements in microscopes, as definite limits to visibility are imposed by the wave-lengths of light, which are absolute factors of the universe. Note (3): There may be " viruses " that do not cause disease, but as we can (at present) know viruses only by the diseases they cause, a hypothetical distinction is pointless.

Viruses have quite recently been obtained crystalline, that is, in indubitable crystals. On being shown such crystals of an infective agent, biologists immediately ask, " Are they

living or dead ? " The answer is remarkable: it is that they are neither, or either, or both, as *you* prefer. Your opinion, and anyone else's, as to whether viruses are living or dead, is as valid as that of the virus experts (of which I am not one), or mine. Until this demonstration of the crystalline nature of viruses was made, writers about virus used to say: " We do not yet know whether virus is a substance (dead) or an organism (living)," but they said it with the idea that some day the proof that it belonged to one class or the other would emerge. That day has not come, for we still have no criterion of what is living and what is dead. It may never come, or finality on the question may already have been reached on the day that the crystalline nature of virus was demonstrated. I suggest then, that virus is like an abstract border-line made visible: it is itself the border. To show you a tube containing virus in crystal form is like bringing you a glass of real water from Charon's Styx, which you have been brought up to believe is entirely a mythical river.

BOOKS

General Biology.—Good accounts of the structure and functions of the cell and its nucleus will be found in *The Unity of Life*, by H. R. Royston (Harrap; London: 1925. 7*s.* 6*d.*), and in Chaps. XXVIII–XXXI of J. G. Crowther's *An Outline of the Universe* (Pelican Book, No. A 22, Vol. 2). Royston's book also gives a simple account of some forms of protozoa and algæ.

Fungi.—There are numerous books on fungi. One of the best general accounts is that given by R. T. Rolfe and F. W. Rolfe in *The Romance of the Fungus World* (Chapman & Hall; London: 1925. 12*s.* 6*d.*).

Bacteria.—Fred W. Tanner's *Bacteriology* (John Wiley, New York. Chapman & Hall; London: 1937, 3rd edn. 17*s.* 6*d.*), includes some discussion of all classes of microbe in spite of its title.

Bacteriology, by H. H. Conn and Harold W. Conn (Williams & Wilkins Co.; Baltimore: 1924. 18*s.*), shares with Tanner's book the claim to be considered the best introductory book on general microbiology. Both these books discuss all classes of microbes to some extent—not only bacteria.

The excellent new work of L. D. Galloway and R. Burgess, *Applied Mycology and Bacteriology* (Leonard Hill; London: 1937. 10*s.*), can be cordially recommended to those—even beginners—who would like to know about the distinguishing characteristics of the different types of bacteria and fungi, and about the uses and control of bacteria and fungi in modern industry, such as dairying, the textile trades, and water supply.

The fairly advanced biologist will like *Microbes and Ultra-Microbes*, by A. G. Gardner (Methuen; London: 1931. 3*s.* 6*d.*). In his text, Gardner takes *microbes* to mean *bacteria only*, without explanation or apology; he discusses no other kind of microbe.

Other Microbes.—There is no book dealing in simple fashion with algæ, lichens, or viruses. What appears to be a rather amusing book about protozoa has recently been announced in America; I have not seen it, and think it deals only with human pathogenic protozoa. There are " hand-books " of algæ, but these are species-descriptions intended for the specializing amateur, and do not furnish connected reading. There are numerous works on natural history dealing with pond life and so forth, but the writers either ignore the microscopic forms of life, or deal with the larger forms in detail to the exclusion of the smaller, such as the bacteria and microscopic algæ.

I am sometimes asked to recommend a book on soil bacteriology. The book, by F. Löhnis and E. B. Fred, *Textbook of Agricultural Bacteriology* (McGraw-Hill; New York and London: 1923. 15*s.*), is interesting, and is

amply illustrated by good coloured plates, but I am not sure that it is still in print. The much more advanced collection of monographs by Sir John Russell and several other Rothamsted authors: *The Micro-organisms of the Soil* (Longmans, Green; London: 1923. 7*s.* 6*d.*), is comprehensive, but rather out of date. For those who can read German, A. Rippel's *Vorlesungen über Boden-Mikrobiologie* (J. Springer; Berlin: 1933. About 6 R.M.), offers what is probably the most concise introduction available. Apart from these, the only treatise on soil microbiology that I can recommend to the general reader is that formed by Chapters V and VI (pp. 341–481) of Sir John Russell's *Soil Conditions and Plant Growth* (Longmans, Green; London: 1937, 7th edn. 21*s.*).

CHAPTER III

SHORT OF ETERNITY

" *Can you do Division? Divide a loaf by a knife—what's the answer to that?* "

" Through the Looking-glass."

ALL the microscopically-small forms of life—except the microscopic worm—have one feature in common which it will surprise you to learn. The possession by microbes of this feature is one of the things which led to the proposal that the kingdom of " protista " should be created for the microbes. If only the three kingdoms of animals, vegetables, and minerals are allowed, then microbes belong according to their kind to either, neither, or both of the animal and vegetable kingdoms. Such a state of affairs is bound to grieve the pigeon-holer. But the special and startling feature that is about to be revealed, distinguishes the microbes sharply from the undoubted plants and animals. Whereas plants and animals die, *microbes are potentially immortal.* When microbes die, it is by some violent death from a cause outside themselves, such as application to them of too much heat or too strong chemicals. Their immortality is somewhat like what ours would be, if we never died of disease or old age, but only by the operation of a catastrophe, such as great drought, flood, or the eruption of a volcano.

Animals and plants start from microscopic beginnings; an animal *in embryo* repeats to some extent the evolutionary development of its race. The microbes have nothing in common with the complex process of reproduction, with recapitulation, that is undergone by higher animals and plants. The microbes have nothing to recapitulate, being already at the bottom of the evolutionary class.

The microbial cell is not only capable of independent existence, but can reproduce itself without any such a to-do as supplies the substance of plots for novels, scenarios for films, and briefs for divorce-court lawyers. The microbe does not invariably have to seek a mate; the bacteria never have to do so. Consequently the bacteria, at least, escape the troubles that accompany and follow courting and wedding. Our news about microbial struggles will be free from reports of seduction, divorce, and *crimes passionnels*. It will all be fit to print.

The higher animals reproduce by addition: $1 + 1 = 2$, and then (perhaps) some more. Microbes reproduce by division, 1 becoming 2. They present the paradox that by dividing they multiply. (If they stay in one place they may become a colony, much as the Red Queen's divided loaf becomes a plate of cut bread. But slices do not grow up into loaves, in the way that divided bacteria grow to be like their originals.)

As you know, in the highest organisms the sexual mode of reproduction is the only one, if you except propagation of plants by cuttings and the like. In the microbes (other than bacteria) a sexual process can alternate with an asexual mode. What you have learnt about the identity of daughter algæ with their mother cells and with each other also holds good, with suitable modification, for bacteria, and for yeasts and protozoa, when concerned in asexual modes of reproduction.

Yeasts divide either into two approximately equal halves, as bacteria do, or else by a mode known as budding. Budding consists of the out-growth of a small blister-like protuberance, which increases in size until it is as large as its parent, being joined with it by a thread-like connexion in the later stages, and then breaks away. One " mother-cell " may form several buds (daughter-cells) at once. Yeasts also reproduce after the conjunction of two cells, but it is not known which is female and which is

male, for there are no obviously distinguishing sexual characteristics.

The higher fungi have many modes of reproduction, sometimes forming a series of changes of considerable complexity; for example, successive generations may adopt sexual and asexual modes. The structures that are formed to carry out reproduction are often elaborate. Fungi can also grow from bits of their mycelial thread-like ramifications, much as some higher plants grow from cuttings.

Protozoa multiply asexually by division, as bacteria and yeasts can, or sexually. It is said that some protozoa reproduce by budding. The division of protozoa resembles that of algæ in that a nucleus is involved.

Nobody knows in detail what it is that induces a microbial cell to double itself by division. A congenial environment (one might say) favours division, but that is merely restating the unknown, for what is there special about the environment that is congenial? Temperature plays a great part, without doubt, in inducing division, and, within rather narrow limits, a rise in temperature leads to more frequent division, other things being equal. This fact is illustrated in the following table. Something very like the same relationships probably holds good in other activities of bacteria, if only one species is present (*i.e.* if we are not dealing with a mixed culture[1]).

Food is very important. Food must not only be abundant but must be available. This last means usually that there must be enough water to make life not merely possible, but easy. I suppose the general proposition that food and drink favour bacterial multiplication will not be questioned. All living things require oxygen for growth. There are other factors, such as the degrees of acidity of the

[1] This reservation is important, but the need for it is not always recognized. A sewage chemist has been known to apply to sewage problems the notion exemplified in this table: that bacteria, of one pure species, about double their activity for a 10° C. rise in temperature. A reading of Chapter XVII will show how unsound the argument may be.

TABLE

GENERATION-TIME OF A (COLIFORM SPECIES OF) BACTERIUM AT VARIOUS TEMPERATURES

Temperature, ° *C.*	*Division occurred about every x minutes; x=*
20	60
25	41
30	29 7
37	17–21
40	17 or slightly more
42	19–20
45	30–34
50	(no growth)

environment. But, given warmth, food, moisture, and other factors to suit, the microbes might be supposed to go on increasing until all the food was used up, and then they would either die of starvation or adopt one of the protective forms which some of them can adopt under unfavourable conditions (many bacteria and some fungi form spores, and some protozoa form cysts, when subjected to conditions adverse to the vegetative form).

If bacteria or yeasts grow in or on a solid medium (substrate), they usually do not migrate far from their place of origin, but remain heaped up (so to speak) as a mass of descendants. Such a mass is called a *colony*; it is very often visible to the naked eye. Later in the book, you will find applications of this " colonial " mode of growth.

In speaking about microbes, " growing " *almost always* means " increasing in numbers, " not " growing in size. " Microbes in a liquid medium tend to grow (increase in numbers) until they have used up all the food, if other conditions are not adverse. It should not be thought that they will go on increasing regularly and rapidly, or necessarily remain uniformly distributed throughout the medium. Microbes may group themselves at one particular level in or on a medium to form a layer or film. This is an index that some condition (such as presence or absence of oxygen) especially suits them there. You will come across

this, also later. Various local and general checks operate —even while food remains abundant—to impede the realization of an " ideal " manner of multiplication. Some of the checks are brought about by the very growth of the microbes themselves. Microbes, indeed, experience effects of population pressure; so do all other living things, including our own race.

Misapplying the fact that under favourable conditions a bacterium can divide and thus provide its equivalent of a human generation, about every half-hour, somebody has computed somewhere that in some very short period—I forget how long—a single bacterium could give rise to a mass of bacteria of greater volume than the whole earth. Similar calculations could, of course, be made for herrings, toads, or any other living things which produce a large number of eggs or otherwise provide a vast number of potential progeny. I could probably find the original of this calculation, and certainly I could work it out, but to do either is not worth the trouble. An unfortunately large number of people accept such calculations as " scientific," whereas real scientists will have nothing to do with anything so far divorced from reality. Also, the truth about bacterial growth is much more interesting than such fiction.

CHAPTER IV

THE PUZZLE OF THE HEATED HAYSTACK

SOIL bacteriologists are painfully familiar with the form of the following conversational opening: " So you work with bacteria ! Germs of disease and so on. How thrilling ! But isn't it dangerous ? " Research work with disease-producing bacteria and other microbes has had much more publicity than other branches of microbiology. For every soil bacteriologist occupied with the amiable aspects of bacteria, there are perhaps fifty workers paid to investigate problems concerning microbes in relation to disease of man, livestock, or crops. This estimate does not take into account the thousands of medical men and veterinary surgeons to whom microbes in general, and bacteria in special, have little but a sinister significance. Few people are aware of the silent service under the feet of the plough-horse and grazing animal, or of the way in which microbes have been harnessed in manufacture.

I have read a number of attempted justifications, written by soil microbiologists, and made with a view to show how useful, and even essential, soil microbiology is. Most of these screeds are pretty dull, and even my professional mind cannot, while reading such learned blurbs, quite cast out the suspicion that it is a professorship or something of the sort which is being held up as the thing that should be sustained. Good heavens, the microbes were at work and had learnt their rôle before man, let alone the microscope, was invented ! Indeed, it was probably one group of soil bacteria—the legume nodule bacteria—that eased the way long ago for first the mammals, and then man, to go ahead beyond the reptiles.

These attempted justifications for a science have had an

air of special pleading, due to the lowness of their aim. They have usually centred on the presumed cash value of microbiology, thus, again, to justify the professor's existence. Stripped of verbiage, the plea becomes: "Please keep on the professorship" (which, with its attendant expenses, may cost £2,000 to £5,000 a year), "and the dividends shall be reckoned in hundreds of thousands of pounds, as here witnesseth . . ." This plea or prospectus is worth just as much as any other promise to pay more than a safe rate of interest.

I shall not attempt explicitly to make any plea for the upkeep of more soil microbiologists, nor shall I try to put a price upon the practical results of the labours of those who have seen into, and harnessed to man's service, microbes from the soil and from other sources. (If I did, I should be careful not to confuse the capitalized value of past achievements with expectations of future rewards.) My aim is the much higher one of taking microbes as a basis for a philosophy.

Uppermost in your mind, reader, is, I suppose, the problem of what use disease-producing organisms are to man—if you are not still puzzling about the meaning of the heading to this chapter. But the value (if any) of microbes of disease to man is not the only point of view to be considered. This is the favourite, if not the sole, viewpoint of the writers of the few would-be "popular" treatises on microbiological subjects. It is, however, only the anthropocentric point of view. There remains also to be considered the value of man, animals, and plants, as hosts to microbes. There also remains to be considered the value of microbes to each other.

You know that haystacks sometimes catch fire. Sometimes the fire results as the result of volition or of carelessness on the part of a human being, who applies a flame or a spark; he thus ends the otherwise immortal lives of some millions of millions of micro-organisms carried in and

clustering on the hay, which we can suppose to be dry. The microbes on the hay have no possible defence against this sort of catastrophe impinging on them by the act of one large organism. Question I put to you is: What is the use of man to these microbes?

Haystacks also take fire "spontaneously," that is "by themselves" and not by the result of man's action (I leave as a philosophical exercise for the reader the problem of the stack set on fire by lightning and other meteoric agencies). The end-result of spontaneous heating is that the aforementioned millions of millions of micro-organisms cease to exist with much the same completeness as if the stack were set on fire by a flame.

The stack that sets itself on fire does so in a curious way dependent at first upon both moisture and micro-organisms. A really dry stack of hay won't "heat" spontaneously; a really damp stack can't be set fire to, but if left alone is almost certain to heat, and to destroy its living contents. The processes that are to consume the living contents and their substrate are started by the microbes themselves. From this microbial initiation, the destructive processes proceed to an inflammatory end, which is peculiarly difficult to forestall. The heating starts inside the stack, where there is moisture and enough food-materials (sugars and other carbohydrates, and proteins) in the hay; towards the middle, too, the hay is well pressed down, so that there is but little air confined between the pieces of hay; there is also no ventilation worth mentioning, and what air there is can be renewed with great difficulty, if at all. Little is known of the early stages of the heating of a stack; the process is almost impossible to initiate on a small scale, and so cannot be studied in a laboratory. Probably the process starts by the growth of miscellaneous microbes (bacteria, fungi, protozoa) which, in growing, use up all the available oxygen; their development then comes to a stop for lack of oxygen, and a new growth starts, composed of the kinds of

bacteria which prefer not to be in the presence of oxygen gas, but to get their oxygen from the breaking-down of substances, such as sugars, that contain combined oxygen. All this growing produces heat, which, on account of the absence of ventilation, cannot be wafted away in the air; nor can the heat be carried off by conduction, on account of the heat-insulating properties of the surrounding hay.

The bacteria that flourish on the combined oxygen of sugars, proteins, and other compounds and in absence of gaseous oxygen, are classed as anaerobic bacteria, or anaerobes. These are in a very favourable situation inside the damp hay, once the aerobes have grown enough to use up all the gaseous oxygen, and have made the central parts of the hay a little warmer. So the anaerobes luxuriate; and why should they stop? The aerobes had to cease growing, because there was only a limited amount of oxygen for them; free oxygen was their limiting factor. No similar limitation applies to the anaerobes. So they go on growing. As they grow, they produce more heat, and the faster they grow, the more heat they produce, and, their multiplication being favoured by warmth, faster still they grow.

Increase of bacterial numbers therefore goes on merrily up to the point where a fresh check to grow begins to make itself felt. This check is the unsuitably high temperature that eventually results from the activities of the bacteria themselves. The check is not sharp, because when the ordinary anaerobes have raised the temperature to such a point that it begins to be uncomfortable for them, a fresh set of anaerobic bacteria begins to increase: these latter are those which flourish only at temperatures high enough to kill the ordinary anaerobes. So, there is a gradual replacement of the warm-living bacteria by those which appreciate real heat; at a later stage, the bacteria adapted only to merely warm conditions are killed, and the so-called thermophilic organisms are left in possession. The thermophilic organisms (" thermophils ") wax in numbers in

much the same way as their predecessors did, until the temperature begins to rise to a degree uncomfortable even to the thermophils. By that time, however, chemical changes that are imperceptible at ordinary temperatures are enabled to take place rapidly. This chemical consumption goes on with the evolution of ever more heat, until the last thermophils are killed. By that time the purely chemical activities have reached a point where there is no stopping them, more and more heat is engendered until the hay begins to blacken, and, driving off the rest of the water, it finally smoulders. If air is brought to the blackened mass, the last requisite for actual fire is provided. That is why a stack does not always catch fire until it is opened in an attempt to save it. The vigorousness of the chemical forces is shown by the observation that small amounts of water poured on heat-blackened hay arrest the smouldering only temporarily; as soon as the water evaporates, the black mass may catch fire again, owing to the seizure of atmospheric oxygen by substances already partly broken down by heat. These pyrolysed substances have been rendered more than eager for oxygen, and, in seizing it, they burn. Control therefore is difficult at any stage after damp hay has been stacked. The only control that can be exercised consists in prevention, rather than cure. The hay should be dry, in which case the microbes merely vegetate for lack of water. If the hay is not dry, it can be salted (salt is added while the stack is being made) whereby the existing water is bound up with the salt, and if sufficient salt is added the salt water becomes an unsuitable medium for the growth of most microbes (no salt-loving thermophile microorganisms are known), or ventilation can be provided, by building air-ducts into the stack, by turning the hay frequently so that it never becomes really hot, or by keeping the stack small. You may have noticed that in Ireland and northern Britain, where the climate is moist, the haystacks are many and small. Now you know why large haystacks

are preferred only in those regions which get enough sun at the right time to dry hay in the field.

But do you know why the successive groups of microbes that prevail in the haystack stage the processes that end in the spectacular suicide of the last and most heat-tolerant group and in the incineration of them all? I don't know the answer, unless it is to say that conditions being right for growth, and the microbes being there—they are compelled (or impelled) to start growing. They certainly have no prevision of the consequences, and can practise no birth-control. If you ask me what good they do themselves. I am quite unable to answer.

Now suppose we consider what good is to be ascribed to disease-producing microbes. If their multiplication in a host—such as man—leads to the death of the host, the problem is very similar to that of the heated haystack. Not all of the micro-organisms in the stack need burn; a proportion may escape on some unaffected part of the stack, and so survive—to start a perhaps more thorough incendiary process in another stack another time. Similarly germs of disease may and often do escape from a patient and can infect a second person, and so on until every susceptible person has had the disease. Owing to sanitary precautions, this does not happen to any great extent in civilized countries. The evolution of epidemics (rapid successive infections of a large number of hosts) is a study in itself, of which I shall not attempt to treat; but I may remark that the results of infection, expressed as numbers of hosts attacked or killed, often appear to proceed through minima and maxima. Either the average virulence of organisms that produce disease changes and alternates with time, or else the average of susceptibility in the hosts is raised or lowered at fairly long intervals. Clearly, a great deal depends upon the relative values of the virulence of the disease-producing micro-organisms and the susceptibility (or its converse, resistance) of the host.

THE PUZZLE OF THE HEATED HAYSTACK

Virulence of a pathogenic (disease-producing) micro-organism, and susceptibility or resistance of its host, are cognate. To impute disease-production to microbes is to consider only one aspect of an impact.

Just as it takes two to make a quarrel, so it takes two to have an invasion, whether by disease germs or soldiers. The term " invasion by pathogenic micro-organisms " is a good one, precisely because it recalls the defence as well as the attack. The possession, by the invaded party, of a defensive armament, has been the aspect that has been most lost sight of by the popularizers of the germ theory of disease. Accent on the body's defences was popular at the beginning of this century, as a result of Metchnikov's observations that certain " white " cells in the blood had the power to mobilize and actively to destroy bacteria that had gained entrance. You will recall that a fragment of a development of this theory of phagocytic defence was a central feature of Mr. Bernard Shaw's play " The Doctor's Dilemma." Since the time of the first run of that play, consciousness about the mechanisms of the body's defence has almost entirely faded from popular thought; one seldom hears references to it now.

Recently the importance of general fitness as a desirable end has been stressed. Good and varied food, adequate housing, exercise, playing-fields, and so on have been widely prescribed as ideals vaguely assisting the well-understood though indefinable condition of fitness. As fitness and disease cannot co-exist, I presume that the campaigns for more milk, more playing-fields, and so forth, are directed towards the annihilation of disease. The germ-theory of disease has not been impugned or questioned by those who urge that more and better food and more recreation assist towards health. The strange thing is that the whole series of fitness campaigns subsist on a presumption: there is no scientific evidence that well-fed (by which I do not mean over-fed) sportspeople are less liable to succumb to invasion

by pathogenic microbes than are the under-fed or the overworked. The organizers of the fitness campaigns have been content to ignore the microbes " causing " disease, and to assume that a population living under good conditions need not fear microbial disease.

I cannot doubt that this attitude is the correct one, but I say so as a matter of faith, not from satisfactory evidence. There has been no investigation of the degree to which a host's resistance to microbial invasion can be raised by good food. There have been a few investigations of the kind that have shown an increase of weight and height in school-children supplied with a daily dose of fresh milk, but the evidence that the children have acquired increased resistance to general infection is not clear. I am thus acting as devil's advocate against my belief that not only does increased fitness result from better food, better housing, and more access to sunlight, but that increased fitness connotes a heightened resistance to invasion by pathogenic microbes (except possibly the viruses). You will understand that I am now writing purely as a scientist awaiting the production of convincing data.

A difficulty in the path of those who would wish to measure the gain of resistance consequent upon better conditions of living is that no unit of resistance is available. The unit of attack is apparently all too clear: it is the microbe. The unit of resistance is unknown, for the phagocytes discovered by Metchnikov represent only one phase of resistance, a phase which is not at first called upon in those infections (*e.g.* typhoid) that lodge in the gut before attacking the integrity of the body.

While the prevailing fear of microbes as agents of disease can be expected to subside before the increase of knowledge concerning the means of building up resistance, I can understand, if not altogether share, the public's fear of microbes. It is a legacy from the early days of microbiology: the ideas—both hopeful and fearful—propagated in

those early days are hard a-dying. As an example of the persistence of popular impressions it may be mentioned that the distinction by letters, such as W.C. and S.W., was accorded to the main London postal districts in 1857, but the N.E. district was amalgamated with the eastern district in 1867, and the abbreviation N.E. was officially abolished in 1869. All this happened about the same time as bacteriology was emerging as a science. The finer division of the old postal districts into sub-districts bearing a number as well as a letter was performed in 1917; right up to that time, and for several years afterwards, correspondents quite commonly addressed letters to London, N.E. In 1917, thanks to the publicity which the newer system received, it became evident that what had been called N.E. was E.8, or some other eastern district; nevertheless, until only about ten years ago the use of N.E. had not quite disappeared, and possibly it lingers yet in a few instances. The non-existent N.E. suffix had been before the public for only twelve years before it was officially abolished, but this brief existence gave it a vigorous survival amongst the public for more than fifty years after it had ceased to have any official meaning.

The teaching of the early bacteriologists was directed towards showing that microbes were agents of disease. In the views of such pathologists, it was implicit that once the microbial cause was tracked down, the discovery of the cure would not long be delayed. This has been a false hope in most instances; the treatment of patients suffering from tuberculosis, for example, does not yet proceed by a direct attack on the causal micro-organism. Knowledge about the microbial cause of disease has undoubtedly been of great value, but usually such knowledge has found its chief usefulness in preventive methods, in methods of improved sanitary control, and in diagnosis. That the body possessed a defensive system, able up to a point to repel an attack, was a set of discoveries that came much later. Some of the defences can be strengthened by using preparations of

weakened invaders; these belong to the spheres of active and passive immunization (*e.g.* anti-typhoid inoculation). The medical scientists have not neglected the study of immunization (immunology): (far from it), but they have decidedly neglected to enquire scientifically into what constitutes and maintains ordinary good health. This is probably because the " causal " micro-organism of a disease is something that can be easily manipulated in the laboratory, and also because for the medical man the study of disease has a peculiar fascination which health lacks.

Undoubtedly the greatest contribution recently made to positive health are the biochemical findings about vitamins, and it is established in a general way that sound nutrition impedes the onset of many microbe-borne diseases. Other accessory substances besides vitamins may be active in this respect.

The common swede turnip provides a striking example of the maintenance or failure of health in presence of an overwhelming number of microbes, according to whether a necessary (and until recently disregarded) element is present or absent. Swedes in the field grow in close contact with soil, of which—as you will recall from Chapter I—every saltspoonful contains at least two thousand million microbes—most of which are alive. The job of most of the microbes is to seize upon vegetable matter and to decompose it; this, however, they cannot do if the vegetable matter is part of a healthy living plant. A swede is healthy, because it is immune to all that microbial swarm. Even should it be accidentally damaged in some way, while the plant lives, rotting does not usually proceed very far. If, however, the soil is so lacking in compounds of the chemical element, boron, that the swede cannot obtain the traces of that element which it requires for normal growth, the soil microbes attack the tissues of the swede, and rot them. This is not exactly a disease, since disease is usually regarded as due to a single specific microbe, which is looked

upon as parasitic on its host. The microbes that attack the boron-starved swede are miscellaneous, not specific. In destroying the unhealthy, because boron-deficient, tissues, the microbes are behaving towards the swede as they normally do in the soil towards any decomposable matter (saprophytically). The point is that their saprophytic energies are without avail against the healthy swede organism.[1] It is not known how the healthy swede keeps the swarm of potential invaders at bay, but we do know that a trace of boron has a great deal to do with it. Obviously, the boron, as sustainer of swede health, is immensely more important than the microbes are as agents of " illth."

You will have seen that the event of spontaneous inflammation of a haystack is practically independent of the microbes. Microbes are always present on the hay, but they can do little unless the " resistance " of the hay has been lowered by improper conditions.

If it seems strange to you that a microbiologist should thus publicly belittle the potentialities of microbes, remember that I have not set out to make your flesh creep, nor to put microbes on a basis of dollars and cents in human health or sickness. I want you to study microbes, and to accept your own conclusions about them.

Nothing I have written should be taken to detract from the need for avoiding the cruder forms of infection. But let me suggest that fear of microbes is an old-fashioned bogy, and that the microbial part in disease is not the aspect of microbes deserving of the most attention. Even the worst epidemic leaves survivors, and in the recent typhoid epidemic at Croydon the number of people who drank infected water, or who otherwise introduced typhoid bacteria into nominally susceptible parts of their bodies, must have been much greater than the number of those

[1] This sounds nonsensical, since a healthy swede is one not attacked by disease. The difficulty is one of words. For " healthy " you may substitute " whole", using it somewhat as in the sense implied in: " a whole man".

who contracted the disease. The question that is still unsolved is not why did tens die and hundreds fall ill, but why did thousands fail to get the disease? You will note that it has been left to a non-medical bacteriologist to propound a fundamental question about health.

If I am right in thinking that health derives primarily from good nutrition, then I may go a step further to the plant, which is the source of all food. The plant depends upon the activity of micro-organisms. Instead of following out the argument in detail, I will conclude this chapter with a suggestive quotation.

Sir Frederick Keeble finished three lectures at the London Royal Institution on "The Soil and the Green Plant" with: "Looking back along this long tortuous road I find it difficult to believe that anybody could have been so slow to reach the conclusion to which it leads, or could require so much material on which to base a hypothesis which ought to have jumped to the mind long ago. The hypothesis is that the health and strength of people and their evolution, and the permanence of human societies, depend on the soil and the green plant. The conclusions are that if the world has got on so well as it has with a half-starved vegetation and a hungry soil, how much better might it not get on when these deficiencies are discovered and made good."

CHAPTER V

MICROBES COME ALIVE

I HAVE said two or three times that microbes are essential for the growth of plants in the field. Some of you may be botanists to the extent of having recollections of growing complete plants with their roots in nothing but a water solution of certain chemicals; or you may have raised mustard and cress seedlings on a damp sponge or flannel; or you may be afflicted with a craze for "soil-less growth," under the impression that it is the latest scientific novelty. It is, indeed, easy to grow plants without any microbes being present at all.

Plants can be grown with chemicals only when the chemicals are chosen to supply the plants' food needs in solubilized form. Given a well-roasted mouse or two, baked straw, leaves, and other plant-remains, a few lumps of granite, limestone, and apatite—all sterilized—and access to filtered air and boiled water, how would the plant fare? These things *contain* all that a plant needs, but the plant is unable to make use of ingredients in such undigested forms. Granite, limestone, apatite, and other minerals are broken down into soluble forms mainly by the slow process of weathering, but plant and animal remains are in nature made available to plants through the agency of micro-organisms. Micro-organisms also take part in making minerals available to plant roots.

It is interesting to see for oneself how microbes live and work, but you will understand that the microbial disintegration of a mouse would present a study that would not only be unpleasant, but would not be very instructive.

The most fundamental demonstration that can be done with microbes is to show that they can grow (can increase

in number) if they are alive, but not if they are dead. For that purpose, I have borrowed an experiment from Sir John Russell's little book, *Lessons on Soil*, Chapter VI, "The Dwellers in the Soil." In principle all that is required is to obtain a sample of ordinary soil, bake part of it, and drop a pinch of baked soil and a pinch of unbaked soil into separate flasks or bottles containing a little milk free from living microbes. I shall discuss this introductory experiment at length, and examine some of its assumptions and implications. I shall indicate later how the experiment can be fined down so that the growing of microbes can be more narrowly studied under better and better control.

Take from the garden or field a little not too moist soil, and take out small stones, pieces of root, and other material which is not obviously soil. Have ready a couple of patty tins, and put about a dessertspoonful of soil on each. Bake one of the tins in the kitchen oven. Pour milk to the depth of about half an inch into each of two small flasks or bottles, and boil the milk very carefully for a few minutes, and allow the flasks to cool after plugging them with cotton wool. (A better way to kill the microbes in the milk and in the flasks is to boil water for some time in the flasks, then to pour it out and put in a half-inch depth of milk that has come from a pint or so just boiled for a few minutes in a saucepan.)

Take out the plug from one of the flasks, and, without touching any of the soil itself, drop a little of the baked soil into the flask; label the flask "baked soil," and replace the plug. Similarly drop a little of the unbaked soil into the other flask; label that "original soil." Swirl the flasks a little, to mix the contents, and leave them in a warm place for a day or two. Then, carefully remove the plugs, without putting them down on any dirty surface, and smell the milk. You will probably find that the baked soil has produced no change; the unbaked soil will have

made the milk " bad," so that it has a pronounced smell (Fig. 5).

Microscopic examination will show the presence of globules of fat in both samples of milk, but nothing else in

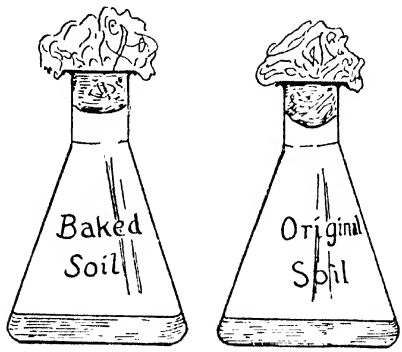


Fig. 5.—Sir John Russell's demonstration experiment with baked and unbaked soil in milk. There is no very evident difference between the appearances in the two flasks, but the contents of one may be curdy.

The picture shows the type of flask most useful for the experiments described in this book: the conical or Erlenmeyer flask. If this is filled to only the extent shown, it allows a large surface of medium to be exposed to air, thus allowing easy access of the microbes to atmospheric oxygen and nitrogen. The labelling of glass and china is conveniently done by writing directly on the surface with a so-called " glass-pencil " (china-marking pencil), such pencils cost about 4*d.* each. They may be obtained in red, blue, or yellow—the last being perhaps the best. With a little practice, writing at least as good as that shown can be done, even on a curved surface, the surface must be highly-polished and *dry*.

the milk into which the baked soil has been dropped. The milk into which the original soil has been dropped will be found to be swarming with tiny creatures among the globules of milk fat. Sir John concludes: " These living

things must have come from the unbaked soil, or they would have been present in both flasks." Living things, similar to those which gave rise to them, must have been present in the baked soil, too, but must have been killed by the heat.

The experiment is based on previous knowledge of how microbes behave. It would be unnecessarily tedious to discuss every point with rigorous logic, but we may mention that essentially two interlocking assumptions are made and tested: (1) that baking both kills and inactivates the microbes; (2) that milk is a suitable source of energy for at least some of the living microbes, so that they can grow and produce characteristic products when given milk. You may be interested to have a discussion of some aspects of your experiment.

It is conceivable that baking might kill the microbes, without putting an end to their ability to bring about chemical change, such as the production of acid, in milk. If that were so, the experiment might fail because similar changes would occur in both flasks. On the other hand, if milk was not a suitable medium for growth of at least some of the microbes present in soil, the experiment would fail because no change would occur in either flask. If the milk had already become sour before boiling, the experiment might fail, because the acid in the milk might make it an unsuitable medium for an expression of development of distinctive soil bacteria to become manifest. If the milk were heavily charged with its own acid-producing bacteria and were not boiled, so that it could become sour during the time suggested for the experiment to last, the experiment would again fail, because such changes as might be due to the introduced soil microbes would be masked by similar or conflicting changes produced by the milk's own microbes.

These arguments are rather academic at this stage. I mention them because I want to show that in microbiology,

as in any other branch of science, nothing can be taken rashly for granted. In searching for new knowledge, experiments are devised to test assumptions about the unknown. The design of experiments calls for clear thinking. Equally clear thinking is needed for the interpretation of the results.

The experiment of baked soil and milk is really a demonstration of the known rather than a search into the unknown. It proceeds from the known facts that baking destroys micro-organisms and renders them inactive, and that milk is a food for many of the kinds of microbes present in any soil.

The demonstration of these facts is very clear, but the experiment tells us nothing about the reason why milk is a suitable medium, for some microbes, nor does it tell us anything about the kinds of microbes that are favoured by having milk—which they normally do not encounter in the soil. One reason for its non-selectivity is the fact that milk is a liquid. The growth of microbes therefore takes place in all directions throughout the liquid: as a result the growing microbes are thoroughly mixed up. Ultimately particular kinds of them predominate, and this predominance after conflict is made manifest to us by the formation of an odour and by a sour taste. Does this mean that the odour-forming and acid-producing microbes are dominant at the time we perceive the results—smell and taste? It does not. Rather, such manifestations as an altered odour, and acidity, announce that the struggle for food and existence is about to enter on a fresh phase. You will recall, from the conflicts in the heated haystack, how each set of microbes predominant at any one time was paving the way for its own extinction and for its succession by a set better able to take advantage of the conditions produced by the previous occupiers. So it is with your soil microbes in milk, or indeed with microbes in any food (medium, source of energy, substrate) in which conditions are not controlled.

Take for example the phenomenon of acid production in your demonstration experiment. The production of acid is brought about by microbes (mostly bacteria). These form acid, chiefly from the carbohydrate (milk-sugar, lactose). Other groups of microbes are liable to produce a varied assortment of substances, such as alcohol, glycerine, and ammonia, from the sugar or from the other constituents of milk. Most of these substances are not acid, but the microbes that produce the non-acid substances do not like acid conditions. On the other hand, the microbes that produce acid are not dismayed by the presence of such neutral substances as glycerine or alcohol in small amounts. Soil added as inoculum to fresh or sterilized milk usually results in the addition of potential acid-producers together with microbes having other potentialities—which, however, they are unable to exercise to any great extent once the acid-formers get really busy. The acid-formers have the further advantage that they have a relatively simple chemical task; they have only to break down the sugar. (This is also true of the alcohol- and glycerine-formers, but they are checked by acid.) The organisms that produce ammonia might in theory produce enough of it for a progressive neutralization of the acid, but unfortunately for the ammonia-producers, their task involves the splitting-up of the chemically complex proteins, by a succession of processes which may demand more time than the acid-producers "are willing" to concede.

Hence it is not long before the acid-producing micro-organisms establish an ascendancy. The faster they grow and the more acid they produce, the less are things to the liking of the acid-haters. The acid-producers do not have it all their own way, of course; a minority of other kinds of microbes are at work and produce their own characteristic products in small amounts. Evidence that something besides acid-formation has gone on is afforded, without chemical tests, by the odour. Lactic acid and most of the

other acids that are formed in the souring of milk have no odour. The odour of soured milk is due mainly to non-acid substances, formed either at the outset before acid-formers have become predominant, or else slowly and under difficulties, as it were, throughout the phase of acid-production.

Now, the acids produced are inimical not only to the non-acid-forming microbes but also to the acid-forming bacteria, after a certain (or better, a slightly uncertain) amount of acid has accumulated. Just as the microbes in the damp hay literally make things too hot for themselves, and have to hand on the torch to a more calefacient and thermophilic set of organisms, so the acid-formers in milk finally are in a state in which their own growth is arrested by their own product(s) of growth. By that time the acid-formers are numerically preponderant, but have shot their bolt, and are compelled to stop growing. I am giving away no secrets in saying that when this happens a considerable proportion of the sugar originally present is still unchanged. What remains can serve as energy-material for a fresh group of micro-organisms, still probably present in small numbers. Such micro-organisms can, at first slowly and under difficulties, effect changes, such as the production of ammonia. The new changes—which usually accompany the decomposition of proteins—result in the setting free of alkaline nitrogenous compounds of which ammonia is the type. Hence, the acid tends to be neutralized, and the stage is set for a fresh struggle. The outcome is always an expression of the balance of the contending groups. If the alkali-producers gain temporarily the upper hand, the amount of free (unneutralized) acid diminishes, to the accompaniment of unpleasant smells due to by-products of the "splitting" (decomposition) of proteins. If alkali-production goes on vigorously, the amount of free acid diminishes far enough to let the acid-producers start growing again, and thus check the alkali-producers. So you will appreciate my

saying that there are balances of conflict. In a substrate of mixed composition (milk) with a mixed inoculum (from soil) the outcome of the microbial conflicts is only broadly predictable. Even after using the most refined means of investigation, the result can be described only for chosen moments at which samples are taken for analysis, and such " snap " samples are inadequate, because the relation of the microbial population to the environment is constantly changing. The conflict is dynamic.

If you keep the soured milk long enough, you may find that its odour has changed for the worse. This will mean that from being merely sour owing to the presence of substances, derived from sugar only, it has had part of its protein attacked and has gone really bad with the formation of evil-smelling products of protein decomposition. The struggle for existence will then have advanced along the stage outlined in the preceding paragraph.

A " clot " may form in the milk, and the clot, if formed, may resolve itself and become liquid again. Clotting is solidification of some of the otherwise soluble milk proteins —mainly casein—owing to chemical change, of which that due to the action of acids is here in question. The resolution (re-solution or becoming liquid) of the clotted casein is not due to mere neutralization of the acid, for the acid clotting is an irreversible process; the casein clot in very stale milk becomes liquid because it is digested, so to speak, and disintegrated, by secretions of microbes which attack protein.

This is perhaps a convenient opportunity to discuss the meaning of the terms " fermentation," " ferment," and " putrefaction." Ferments are the agents that cause fermentation. Fermentation is usually regarded as a process due to microbial activity, hence, " ferment " should equal " microbe." Unfortunately, the situation is complicated by the use, by the French especially, of the word " ferment " for such substances as rennet, which is derived

from the stomach of the calf, and takes its activity from that large animal, and not from micro-organisms. Leaving the case of rennet aside for the moment, we are free to say that "ferment" is a fair, though outmoded, equivalent for "microbe" or "micro-organism."

Since fermentation and putrefaction are both induced and carried on by microbes, we are left with the very proper question whether there is any difference between the meanings of these terms. The answer seems to be that there is no real difference, but that putrefaction implies the state of advanced decomposition of animal or at least protein substances, in which an offensive smell is given off. About a hundred years ago, Liebig sought to distinguish fermentation from putrefaction in a way which we may express to-day by saying that fermentation is microbial decomposition of carbohydrates, while putrefaction is microbial decomposition of proteins. This was a terminological advance in his times, but the distinction is too loose to serve any useful purpose now. You may say, if you like, that your experiment with soil and milk is an example of fermentation preceding putrefaction, but what have you gained thereby?

The "definition" of putrefaction given in the preceding paragraph suggests that fermentation is any process of chemical change produced in non-protein substances by microbes. This is roughly the sense usually accorded to it, though it may be doubted whether many users of the word "fermentation" stop to analyse the meaning or meanings they attach to the word.

Putrefaction is a term usually used in a broad sense, without much qualification. Several kinds of fermentation are, however, recognized; these are distinguished either by the kind of microbe which is the principal agent, or by the type of principal product. This, of course, makes things confusing for the serious beginner, who has to learn to appreciate and sort out the meanings which have accrued to

vague terms during the centuries that have elapsed since fermentation began to be of sufficient scientific interest for men to try to account for it and describe its phènomena. The philosophical history of fermentation is, like any other history, a record of exploded ideas; some of the fragments of shattered notions linger yet and cumber terminology.

Thus, there are the alcoholic fermentation by yeast, that produces ethyl alcohol from sugar (typically grape-sugar, also called glucose; there are other yeasts that can produce ethyl and other alcohols from other sugars): and the lactic acid fermentations by bacteria, that produce lactic acid from milk-sugar (lactose): or, two kinds of fermentation, differing both in agent and in product, but occurring simultaneously, may together be called a lactic fermentation, as in the fermentation of milk by a mixture of yeasts and bacteria which gives rise to the "fermented milks" discussed in Chapter XIII. All very confusing. This sort of terminology worked sufficiently well for a time in the early days of microbiology, when known fermentations were few and their products were supposed to be simple, but as knowledge grew, and a vast range of substances was found to be produced by microbial action, the new knowledge outran the capacity of the old terminology. I mention the old terms here not because I desire to perpetuate them or think they have much value, but because you are sure to have heard some of them, and I feel that you will be disappointed if you do not get some sort of explanation of them. Nowadays microbiologists don't coin new names for every fresh variety of fermentation (except possibly in America, where scientists are generally fond of abstruse-sounding neologisms). I might talk, for example, about the production of butyl alcohol by the action of *Granulobacter saccharobutyricum* on a selected hexose: this may mean nothing to you yet, but because it is exactly expressed, it gives you more chance to understand what I mean than if I were to speak of a butylogenous fermentation—a term which can

carry only an irritating vagueness to a trained microbiologist.

You will not object to my reminding you that I am much more wishful for you to grasp some of the philosophy of microbiology, and, by extension, of science in general, than I am to impress upon you isolated facts about the size, shape, or behaviour of particular microbes. Those facts, after all, are best kept in the textbooks, and only the perspiring student need bother to *remember* them; the specialist can always look them up.

There is, however, one term which is very relevant to your demonstration with milk and a pinch of soil, and which I should like you to learn, understand, and use: it is "enzyme." When I wrote, in this chapter, and earlier in the book, about microbes attacking, decomposing, splitting, or digesting plant material, sugars, proteins, and so on, did you feel like asking how the microbes performed their attack and digestion? You will have recalled that they have no teeth or limbs or claws; bacteria and fungi have no stomach or visible digestive organs. Animals digest solids after ingestion, but a bacterium never swallows a particle: all its food is fluid. How then can a bacterium or even a host of bacteria digest a lump of casein clot, or break down a mass of fungi in soil or a compost heap?

The answer is that digestion in this sense (for which there are more precise terms with which I shall not bother you) is performed by means of secretions that exude from the living microbe and act upon the solid food from the outside —outside the microbe, and from the outside of the solid. These secretions are called enzymes. They are not confined to microbes; in fact the best-known enzymes are probably two which occur in the digestive tracts of large animals, their presence being independent of the microbial population of the gut; these are pepsin and rennet (rennin). Enzymes are common in plants: the characteristic tastes of mustard, horseradish, and bitter almonds are due to the

activity of the enzymes, which split up originally bland substances, and release a chemically simpler substance powerfully affecting the nerves of smell and taste. Enzymes cannot act without water; hence one reason for the necessity of moisture for microbial activity.

You will get a very striking demonstration of this extracellular digestion of a protein by an enzyme if you try to make a pineapple jelly from gelatine and a *fresh* pineapple. Leave it long enough, and the jelly becomes liquid, at first near the pieces of fruit. You may indeed have made the experiment involuntarily, perhaps believing in the superior dietetic value of fresh over tinned fruit! Well, this is a case where the canned product scores on one count at least. The explanation is (1) that the fresh pineapple contains a gelatine-digesting enzyme, and (2) that the enzyme is destroyed by the heat applied during canning. Most enzymes are destroyed (inactivated) when their bearers are heated to about 60–70° C. (140–160° F.). Hence when you baked the soil, you did more than kill the microbes; you also killed their contained enzymes. It has been suggested that the enzymes of dead bacteria are capable of activity—provided that the enzymes have not been inactivated by heat or other agency; but the suggestion has not been fully confirmed, and I need not do more than mention it.

The concept of microbial enzymes has been very useful to microbiology, as it has enabled some precision to be brought to ideas regarding fermentations. Just as microorganisms have been called "ferments," so enzymes have been called "unorganized ferments"—unorganized, because not living. In fermentations caused by microbes, the active agents are really the enzymes of the microbes. There are "fermentations" which proceed under sterile conditions, that is, in the total absence of microbes; the majority of enzymes of the higher plants act without the intervention of microbes, as in the cases of the production of pungent substances in mustard, horseradish, or bitter

almonds. Enzymes can exist without microbes, but, without enzymes, microbes cannot begin the chemical changes needful for their growth and reproduction. Enzymes are the means whereby foodstores are made accessible in the microbial struggle for existence: they are the currency of the microbe world.

BOOK

Lessons on Soil, by Sir E. John Russell (Camb. Univ. Press : 1926. 6*s*.).

CHAPTER VI

MORE ABOUT GROWTH

"I never ask advice about growing," Alice said indignantly.
"Too proud?" the other enquired.
"Through the Looking-glass."

TOWARDS the end of the last chapter, I realized with surprise that I had written a very long disquisition around and about what seemed a simple experiment. By the time the end of the chapter had been reached, I felt that the disquisition was far from being unwarranted. It has enabled you to gain what is probably a new concept, namely, that of enzymes, but the instillation of facts is not my chief purpose.

The introduction of a complex microbial population from a pinch of soil into a complex yet ill-defined substrate such as milk may be a simple method of showing that microbes set up chemical changes. Milk is only less complex than the mouse which I invoked early in the last chapter. The use of a disinfectant instead of milk is a simplification. Yet neither of these essays in microbial growth is well adapted for answering the more searching questions: What microbes? and What alterations? To get more detailed information about microbes, you need to let them practise on simple chemical substances of known composition. You will then learn by growth-responses of the microbes whether they like, that is, can attack, the known substances; secondly, by studying the chemical changes, you can get some further precision of knowledge.

You may at this stage apprehend the dawn of a classification of microbes based on the foodstuffs they prefer or require. Thus, one microbe will attack substance A, but will be found to be indifferent to substance B. Another

kind of microbe may like both A and B, but be indifferent to C, which is attacked equally well by the first two kinds of microbes. Still another may attack both A and B, but may grow well whether C is supplied or not, being apparently able to supply C for itself. This sort of classificatory testing might be applied almost indefinitely if you had a big choice of chemicals on hand, but you will at once grasp the fact that you could not in any case hope to distinguish one kind of microbe from another by such tests unless your microbes were already sorted out for you into single kinds—species, the biologist calls them. Now, wherever your soil comes from, it is very far from being a pure culture: it is a highly heterogeneous mixture, not merely of species, but of bigger classes: it contains animals and plants; amongst the latter alone being algæ, yeasts, fungi, actinomycetes, and bacteria. Probably a trained microbiologist could easily recognize at least half a dozen species as belonging to each of these five classes!

However, the apparent vicious circle can be broken. In Chapter VIII you will learn how to prepare one species of microbe in substantially pure culture—that is, containing substantially one species. Your "isolations" will not be rigorously pure, but will do to go on with, and later on you will learn how to obtain a genuinely pure culture.

When learning about microbes, it is desirable to do experiments which have been simplified far enough to give an essential feature a chance to stand out. It is allowable—and, indeed, highly desirable—to use chemical substances as nutrients for the microbes which it is desired to cultivate, for only by using known substances can we ensure standard conditions of nutrition. We thus control, more or less, the outcome of the microbial struggles.

It is a microbe or a group of microbes whose behaviour we wish to study. We must assume that the behaviour of the microbe is not known to us beforehand, that it is unpredictable, in fact. If everything else—temperature,

moisture, food, and so on—is made constant, the microbe supplies the sole variable factor, or *variable* for short. If the food is of uncertain composition, or if temperature fluctuates widely, a number of other variables are introduced thereby, and the drawing of a valid conclusion from the experiment may be defeated.

One more caution or what-would-you-call-it before you take your notebook and pencil and set off on your initiation into reportorial work amongst the teeming microbes. It is: not to take the heading or headline of the last chapter too literally. Microbes cannot come alive! If they can be induced to live, it must be that they are already alive. I have given you a sensational headline, that's all, just to exemplify the Fleet Street frame of mind—now for that pencil and notebook of yours. You will not be merely a reporter, however, because you will also have to be a manipulator; the fate of millions will be in your hands.

CHAPTER VII

THE MICROBIOLOGY OF DISINFECTANTS

" *You've no right to grow* here," *said the Dormouse.*
" Alice in Wonderland."

I'M afraid this title sounds rather forbidding after our light-hearted start. The headings of the earlier chapters might almost be headlines, but this one sounds something like Science at its worst, and something like nonsense.

The experiment in Chapter V was not really very simple, because you had the trouble of sterilizing part of your soil by baking it. You also had to boil some milk. The experiment was a demonstration of what you already know, probably, but it did show you that dead microbes do not effect changes in a substance so rich in varied microbial foodstuffs as milk is. You did, however, learn something from the experiment itself, and, to the facts you gleaned, I added a good deal more, not derived from your experiment. You learnt, too, that soil is a source of microbes which is very handy for the beginner in microbiology.

But you don't know what microbes you were using, nor do you know exactly what sort of chemical substance they were attacking. Milk contains three chief sorts of organic substances : protein, carbohydrate, and fat. I may add that the appearance of sourness (production of acid) rather suggests that the carbohydrate was chiefly attacked, but, as I pointed out, the changes may go further. So the actions of the various microbes on the various constituents of milk were inextricably entangled.

Later on, you may like to try the effects of a single kind of microbe on a single kind of foodstuff, such as a sugar, but that will be much later, I think. Also that sort of

experiment would not be highly instructive by itself, and is moderately difficult to do properly.

In this chapter I am going to make use of some facts you have already learnt, to show how you can make conditions considerably more simple than they were in the demonstration in Chapter V. You will still use soil as a source of microbes (I may as well say "as an inoculum," for that is a useful term). You must not bake the soil, now, for all the knowledge we can usefully get out of doing that has been garnered. You had to boil the milk, you remember, because there are usually live microbes in milk. It was necessary to boil the milk to kill as many microbes as it was practicable for you to do, in order that your demonstration about the living microbes in the soil should not be spoilt by confusion arising from living microbes already in the milk. We can avoid that complication, and also avoid the complication introduced by the complex chemical make-up, if you adopt my suggestion of using a disinfectant to make your microbes grow.

It is reasonable to assume that there are no living microbes in the concentrated disinfectant, but even that need not be taken on trust, for you will find that there are two strengths at which your chosen disinfectant will not allow microbes to grow: namely, when it is too strong for them, and when it is too weak. You may use any disinfectant that is solely a coal-tar distillate: crude "carbolic acid," "cresylic acid," etc. These have certain manipulative disadvantages.

There is a proprietary disinfectant made in England which is well adapted to your purposes. It mixes readily and completely with water, and thus avoids one bother; it is essentially, if not wholly, a coal-tar distillate, containing about 40 per cent. of "total phenols." As this preparation contains only one type of active substance—namely, the phenols—its use is more instructive for you than the use of milk would be. You may use chemically

pure carbolic acid if you wish. This is true *phenol*, and is the type-substance for the others. Whatever you use, don't get it in concentrated form on your skin.

Proceed as follows: Mix thoroughly a full teaspoonful of your chosen substance with a pint (or a litre) of water. Have ready in a row a number of clean vessels, such as cups or glasses, and a small clean cup, wineglass, or other vessel holding a little less than half as much as the others do. Put as much water as the small cup will hold into each of the larger cups in a row, except the first. Put two small cupfuls of the dilute disinfectant into the empty cup; now pour out enough of the dilute disinfectant from the large cup into the small one (which you have just used) and pour from the small cup (now full) into the one which has water only, and stands next to the cup with the disinfectant in it. Mix *well*, and pour a small cupful of the mixture into the next cup, mix, and repeat all down the line. You then have a series of, say, eight or ten cups, each containing one small cupful of disinfectant solution progressively weaker along the row; each cup has twice as much disinfectant as the one has that comes after it.

Now take a small teaspoonful, or a large pinch, of soil, and free it from stones, rootlets, and obvious rubbish. In a pint or more of water dissolve a small teaspoonful of sulphate of ammonia and half a teaspoonful of potassium phosphate. Shake the soil vigorously with the solution in a wide-mouthed bottle, stoppered, of course. After about a minute's shaking, let the bottle stand for a few minutes to deposit sand, etc., in the bottom. Pour off the turbid liquid into a jug. Immediately fill your first small cup (cleaned) from the jug, and pour the contents of the cup into the disinfectant solution at either end of the row. Swirl or mix the contents of the jug, fill up the small cup afresh, and pour that cupful of inoculum into the next large cup. Inoculate every cup in the row in the same way.

Smell your cupfuls, and note your findings; then cover each cup to keep dust out, and to prevent the disinfectant (which is rather volatile) from evaporating. Of course, it is better if you can use small flasks, and plug them with cotton wool. Glasses are better than cups for your purpose. In any case, don't have your liquid *more* than halfway up its container.

You will then have a series of dilutions. Though you may not know accurately the strength of solution in the first cup, you can be assured that the strengths in succeeding cups will be in the ratio of 1 to 2. If we call the strength in the first cup *d*, the next will be $\frac{1}{2}$ *d*, the next $\frac{1}{4}$ *d*, the next $\frac{1}{8}$ *d*, and so on. The tenth cup will contain disinfectant only about one-five-hundredth as strong as there is in the first, and one-thousandth as strong as the first solution you made. Keep your vessels (labelled *d*, $\frac{1}{2}$ *d*, etc.) in a comfortably warm room, and watch what happens. In a very few days you will see that the contents of the cups about the middle of the row are going brown, and are ceasing to smell of disinfectant as they formerly did. The exact position—and, indeed, all your findings—will depend a good deal upon what disinfectant you use, upon the amount of sulphate of ammonia, and upon other factors (Fig. 6).

At the start, however, you will still have some of your first lot of solution, of strength 2 *d* left; you may like to repeat the experiment with another series of cups inoculated with soil from another source. I would suggest that it would be sounder training for you, if you set up another (or even two or three more) series of cups, inoculated with the *same* soil treated as nearly as possible in *the same way* at the same time. Or, you could repeat the sets of cups, and inoculate every one with soil suspension from one large lot. This repetition two, three, or more times is called *replication*, and your similar sets (or individual cups having the same treatment) are called *replicates* or *parallels*. If you do not replicate (repeat at least once) you have no check

on your results; whereas, if you do replicate, your parallels will either confirm each other, or (if their behaviour is different) will give you an idea of your experimental error, if that is reasonably small; and, should the behaviour in corresponding cups be *widely* different, it will suggest that some factor has not been taken into account. Therefore, before you start comparing various soils of unknown behaviour, make sure that your technique is sufficiently

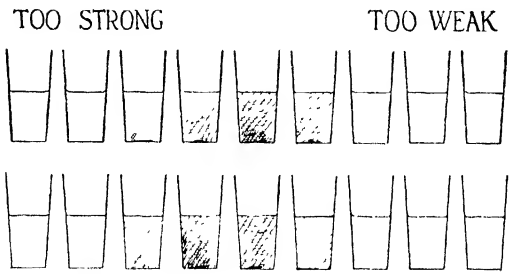


Fig. 6.— Showing the sort of variation that can be expected after a few days in two parallel sets of dilutions of a coal-tar disinfectant inoculated with a soil suspension The actual position and extent of the observed alterations will depend upon the dilutions used. The algæ may be expected to appear earlier in the lowest concentrations of disinfectant than in the middle vessels.

accurate to give you consistent results with *one* soil; that is, see whether you can get browning to occur in parallel cups (containing identical amounts of disinfectant) at about the same time. You will then have varied only *one* factor at a time: namely, the concentration of the energy material. Your error should not exceed one cup either way; that is, one parallel may be brown, while its fellow is not, but both parallels on either side of it should be brown or not brown. That is a fairly generous margin of error. It is calculable, too.

What does the browning mean? It means that some microbes in the soil have so much liked the energy material you have provided for them that they have attacked it, and have broken it down into some other substances. Obviously the disinfectant has changed; it has lost its smell, and changed in colour, and produced some brownish material insoluble in water. This change is the sign of microbial attack. I may add that the brownish material is not unlike humus, which is a complex material, here produced by recombination of products of bacterial breakdown of the phenols (Fig. 6).

At one end of your series the disinfectant will not have changed: there it is lethal to the microbes. At the other end it will not have appeared to change much, because there is not enough of it to undergo visible change, or to serve as food for many microbes. If there is marked change in the "weakest" cups, it is because your soil suspension was too strong, and you have thus added an appreciable amount of energy material of some kind with the soil.

The disinfectant, then, has been the main energy material for the microbes. If you look at the browned liquid with the aid of a good microscope, you will see many live bacteria in it. The sulphate of ammonia and the phosphate were intended as sources of nitrogen, phosphorus, and potash, which microbes (like plants) require, while the disinfectant served as decomposable organic material.

This experiment is rather nice because it gives you both an upper and lower limit of concentrations for visible microbial growth. (I may add that the microbes that like phenolic energy materials are nearly all composed of bacteria.)

If you keep the cups for a few weeks exposed to the light (that is why glasses are best) you will see another change: a green growth will develop on the sides of the vessels which have had all their disinfectant transformed by the bacteria. (After that has happened—as judged by the nose test or by

the appearance of browning, it is legitimate to leave the covers off the cups.) This green growth is composed of algæ, and is evidence of the entire disappearance of the phenols—as such.

You could make a similar experiment with sugar instead of some form of carbolic, but with sugar you would have got growth at every concentration except the very weakest, and, besides, you might have introduced an appreciable number of microbes with the sugar, unless it were sterilized. In the experiment as I have given it, there is no need for any sterilization, unless you want rigorous proof that the microbes do not come from some particular source, such as the cups or the water. I don't think you need bother.

There are all sorts of instructive variations on this experiment. The chief thing to learn is the importance of doing parallel (replicate) experiments varying only one factor at a time. Once you have grasped this, you can compare the effects of different soils on sets of dilutions made up in exactly the same way; for this, you will require to measure your disinfectant and solutions fairly accurately, and to weigh your soil, or your results will not be comparable and will be of no value. You can also compare the bacterial energy-value of different disinfectants, using one soil as inoculum for all- and be sure to use the same weight or measure of each substance you test. You can also try the simpler experiment of setting up parallel experiments with and without, say, the sulphate of ammonia, to see what happens when no nitrogen is added (or, if you like, whether your soil is rich enough in nitrogen to allow of microbial growth under the conditions of your experiment). For this you will need at least four sets of cups: two replicates with, and two without, sulphate of ammonia. A still simpler test is to compare the effects of adding disinfectant to soil suspension, with the effects obtained by adding soil suspension to disinfectant as I first suggested. If you want to test two factors at one time (say, two soils with and

without adding sulphate of ammonia) you will need at least eight sets of cups. You will see that an experiment to test many factors (" to ask many questions ") at one time is perfectly possible, but must necessarily be tedious, if not complicated. The possibilities are, however, endless.

Nobody, as far as I know, has tried the effect of microbes on coal-tar disinfectants containing soap in solution (the lysol group), or on other organic proprietary disinfectants. Would you like to be a pioneer, and try to grow microbes on some of the widely advertised organic disinfectants ? [1]

A more important subject for investigation would be the anaerobic decomposition of phenols. The decompositions I have suggested for you are easy to do because they are performed in presence of air—that is, aerobically.

One thing more. That you can do this experiment at all is due to the research work of numbers of people who investigated the unknown, and found that there were microbes that could live on such apparently queer substances as phenols. The story of their work is interesting in itself. It belongs to history now, and the results of the work to you. My predecessor and my present Head were two of those who did such research, and they would be the first to acknowledge that *their* work depended upon the discoveries of others before them, and so on.[2] That is how science grows; and the discoveries of one decade are often the material of elementary experiments in the next.

[1] Such trials will be interesting, but can hardly be called research; they seem to involve no new fundamental principle. I present the idea to any young student who may be looking out for easy methods of compiling sufficient data for publication in a paper. If he finds out the effect of soap on bacterial proliferation in presence of phenols, that will be something. I warn him, however, not to overlook the factor of alkalinity.

[2] P. H. H. Gray and H. G. Thornton, " Soil bacteria that decompose certain aromatic compounds," *Zbl. Bakt.*, Abt. II, 1923, **73**, 74 [23698]; Mrs. Annie Matthews, " Partial sterilization of soil by antiseptics," *Journ. Agric. Sci.*, 1924, **14**, 1 [10966].

THE MICROBIOLOGY OF DISINFECTANTS

We now know that it is not really queer that microbes should like coal-tar disinfectants as food, for any organic material is decomposable by some microbe. Phenols are excreted in traces in urine, and if they were not got rid of by some agency they would accumulate to the point of being poisonous to plants. The soil microbes that decompose phenols are therefore scavengers.

CHAPTER VIII

SOME MICROBES COME UNDER CONTROL

"I'm glad they've come without waiting to be asked," she thought.
"I should never have known who were the right people to invite."
"Through the Looking-glass."

ONE of the most fascinating ways of making the growth of microbes visible is also among the simplest. Like the experiment with disinfectants, this requires no special apparatus; no sterilization; but it requires a few additional cheap and harmless chemicals. It consists of making a few mud pies, and adding to them certain chemicals to serve as food. The response of the microbes—or their lack of response—is shown by their growing, or not growing, as the case may be, and so it is possible within limits to ascertain their likes and dislikes. Unless you count the making of mud pies to be a disadvantage, the only disadvantages of the technique are (1) that it "works" only with one species of bacteria, and (2) that if that particular species of bacteria is absent from the sample of soil you select, there will naturally be no growth. Neither objection is very serious. The first makes it sound as if the experiment were very unlikely to succeed, but in point of fact the bacteria which the experiment is designed to grow are so very widely spread, that the chance of missing them is small. Objection No. 2 is more real, at least to the beginner, who may be disheartened by a series of "failures" to make the bacteria grow, due to no fault of his or her own. However, by casting your net wide to include a number of soils from different localities, you are almost bound to succeed after a few tries, supposing you do not succeed at first. The experiment is so very easy that you may be almost assured, before you start, that a failure will

be due not to any fault of your manipulation, but to the absence from your sample of those bacteria upon which the test depends.

This is what you will need for the simplest form of this experiment. *Potassium phosphate* (dipotassium hydrogen phosphate); *acid potassium phosphate* (potassium dihydrogen phosphate); *mannitol* (this is a sugary, solid substance chemically related to ordinary alcohol, but is not intoxicating; it is a carbohydrate, which the bacteria use as energy material); precipitated chalk. All these are harmless, cheap, and easily obtained in small quantities from your druggist. The mannitol is the most unusual. If you cannot get mannitol, you may use starch instead. The phosphates and mannitol should preferably be powdered, but that is not essential: the starch (if you use starch) must be powdered, and should preferably be rice starch, since that is the kind with the smallest grains.

You will also need a solid nickel spatula or an old nickel-plated spoon, a microscope-slide, or other small piece of smooth clear glass without sharp edges; and some small glass or china pots (ointment pots do very well, though pill- or match-boxes can be used at a pinch). The pots should preferably be about an inch in diameter and about half to three-quarters of an inch deep. The number you will need will depend upon the number of simultaneous experiments you want to make: two is the minimum, four are better: and as many more pairs as you like.

The experiment consists in outline of this: *Azotobacter* can be induced to grow visibly on the surface of a moist soil, if it is present in the soil and if it is supplied with carbohydrate food-material such as starch or mannitol, and is also supplied with a sufficiency of phosphate and potash. As regards its need for phosphate and potash it rather resembles the higher plants. (Hence its use in soil analysis, later.)

Azotobacter does not like an acid medium; it prefers one that is neutral or slightly alkaline.

If the soil is acid, you can correct that by adding a little chalk, just as the farmer adds lime or chalk to his " sour " land. The two potassium phosphates will supply potash and phosphate, but since one is rather strongly alkaline and the other is rather strongly acid, a mixture of the two must be made that is approximately neutral.

Method.—Mix intimately two parts by weight of dipotassium phosphate with one part by weight of potassium acid phosphate. Make a solution of one part of this mixture with a thousand parts of distilled water or clean filtered rain water. That is, 1 gram of the mixture in a litre of water, or 70 grains of the mixture in a gallon of water. If you prefer it, you may add 0·67 grams (47 grains) of the dipotassium phosphate and also 0·33 grams (23 grains) of the acid potassium phosphate directly to a litre (gallon) of water. The solution should be made at room temperature and allowed to stand for a few hours; you should shake it after making and just before use. If you are in a hurry you may use gentle heat to ensure that the salts are completely dissolved, but then you must cool the solution under the tap before using them. No boiling or sterilization is necessary.[1] Reserve a little of the water (boiled or not depending on whether you have boiled the solution, pots, etc.) for later use.

The one-tenth per cent. solution of potassium phosphate which you now have, will be neutral, and will not make the soil, to which you are to add it, more acid or alkaline than it naturally is. If you wish, test your solution for acidity

[1] Unless you ask from the experiment that it should give rigid proof of the presence of *Azotobacter* in the soil. It is possible, of course, that by using unsterilized materials and apparatus you may be introducing *Azotobacter*; if you therefore decide to boil the solution in order to kill *Azotobacter* present in the water or the salts (*Azotobacter* in water being easily killed by boiling it for five minutes or even less) you must also sterilize everything else you use (especially the pots), or your pains will be wasted as far as supplying a rigid proof is concerned.

or alkalinity by adding a couple of drops of the Bo... Drug House " Universal Indicator " to a test-tube about a third full of the solution. If a greenish colour is shown, there is neither acidity nor alkalinity: in chemical language, " the reaction is neutral." If, however, the reaction is acid (shown by a yellow, orange, or red colour) or alkaline (shown by a bluish or blue colour) something is wrong, either with your water or with your salts. In that case it would be best to make up the solutions of the two phosphates separately: 0·67 grams (47 grains) of the alkaline phosphate in half a litre (half a gallon) of water, and 0·33 grams (23 grains) of the acid phosphate in another half of a litre (or half-gallon) of water. Test each solution with the indicator to see whether it is acid or alkaline—the redder the colour, the more acid, and the more blue or violet the colour, the more alkaline—and then mix small equal lots of the two solutions in various proportions until you find by trial a mixture that has the correct neutral reaction giving a light greenish colour after a drop or two of the indicator solutions has been added to a small test portion.

This method of making and mixing separate solutions of the two phosphates is the only one you can reasonably adopt if you have neither distilled nor rain water, and are obliged to use tap water. Tap water nearly always contains salts of lime, and other substances, which combine with the phosphates and thereby often produce a cloudiness due to precipitation of finely divided insoluble phosphates of lime and so forth. The cloudy precipitate in the tap water will not matter very much in itself, but its formation may alter the reaction from the desired neutrality. Moorland waters do not always form such a precipitate, but sometimes contain traces of peat acids, upsetting the reaction in another way. Hence, if you use tap water, make separate solutions of the phosphates, and mix them by trial, testing for neutrality, until you get a neutral solution, then for future reference note the proportions of each solution.

You may assume that in any one district the composition of the tap water will not vary significantly at different times.

The soil should be fresh, not unnaturally wet. It may also be air-dried. If fresh it will contain 15–20 per cent. of moisture; if air-dried it will contain at least 6–7 per cent., even though it is dusty. Pass the soil through a coarse sieve, spread it in a thin layer, and pick out stones, bits of root, and visible foreign matter.[1]

Weigh some of your soil (or squeezed pond mud) roughly, and add to it a hundredth part by weight of mannitol or one-twentieth part by weight of starch. Label this M.

An equal portion of soil is reserved without mannitol or starch. Label this C.

Mix a little of C with enough distilled or rain water (boiled, or not, as the case may be: see p. 90) to make a stiff paste. Fill one pot with the paste, and smooth off the top with a glass slide. Label this CO. This is the control, because it receives no treatment at all, except mixing with water.

Now mix a little of M with water similarly, and label this mixture MO.

Now treat some of both C and M (separately) with enough of your neutral phosphate solution to make a paste, using the phosphate solution instead of the plain water used for CO and MO. Label the resulting pots of soil paste MP and CP (P standing for phosphate).

To portions of C and of M add a *little* chalk, and mix each well. Treat these with plain water (CLO, MLO): L stands for "lime," since chalk is carbonate of lime: and with the phosphate solution (CLP, MLP). Farmers speak of liming whenever they mean using lime in any form, whether as quicklime, slaked lime, or carbonate of lime (chalk and limestone).

[1] You may also use pond mud, but if it is very wet (as it probably will be) it is best to squeeze it to remove excess moisture.

You will now have the following set:

No Carbohydrate		*Carbohydrate*	
no addition:	CO	no other addition:	MO
+ phosphate:	CP	+ phosphate:	MP
+ chalk:	CL	+ chalk:	ML
+ chalk and phosphate:	CLP	+ chalk and phosphate:	MLP

You should, however, have more than one pot of each. It is much better to make two or three pots of each treatment, because if there should be no growth of *Azotobacter*, you would put down the result as a negative response, whereas it might be due to some accidental failure or omission. To safeguard your *interpretation*, therefore, it is desirable to *replicate* the treatments several times at once, from each lot of soil.

The above set of eight treatments includes every possible combination of the three factors: carbohydrate, phosphate, and chalk. There are eight treatments, because one factor demands two treatments: without and with: two factors demand each of these twice (four treatments), and three factors demand twice four treatments ($2^3 = 8$).

As each treatment should be replicated at least twice, and better three times, a single 3-factor experiment will need 16 or 24 pots, while a 4- or 5-factor experiment would need twice or four times that number.

However, I shall not here enter further into the design of factorial experiments. It is a very new subject, to which I shall refer later.

Having prepared your pots of moist soil, put them into a warm place, and to prevent undue loss of moisture, cover them lightly with an inverted box or dish not touching the smoothed surface. The bacteria do not require light for growth to occur. Keep them from two to eight days. Examine the surface for growth of pearly rounded colonies, at first discrete, but probably confluent later. These will be colonies of *Azotobacter chroöcoccum*. Moulds and other micro-organisms may also grow, but very frequently the superficial growth is apparently pure *Azotobacter*.

When growth occurs, it means that *Azotobacter* was present in the soil (or, less probably, was added with the water or other materials). Nothing more was needed than for you to make the conditions right for it to grow into visible masses.

APPENDIX

In an experiment with *Azotobacter*, similar to that which is described in Chapter VIII, the colonies on mud pies were counted with the aid of a hand-lens —all within an hour or so—and the following results were obtained (three parallel pots of each treatment, carbohydrate having been added):

Treatment	*Number of colonies*		*Increase or decrease of averages from control average, actual*	*Percentage of control*
	Actual	*Average*		
None (control) .	36, 41, 43	40	—	100
Chalk only	32, 38, 42	37 3	— 2·7	93 2
Phosphate only	40, 43, 46	43	+ 3 0	107·5
Chalk and phosphate together	42, 45, 49	45 3	+ 5 3	113·2

There *is* a decrease from adding chalk; there *is* an increase from adding phosphate; there *is* a further increase from adding both. The increase from adding phosphate is only slightly larger than the decrease from adding chalk, and the increase from adding both is relatively quite large. These are *facts*. What are the conclusions? In particular, should the farmer who owned this soil buy both lime and phosphate? It is impossible to tell—either from looking at the original counts (which are fairly consistent within sets of parallels),[1] or from the averages (actual or percentage). Nobody can do more than guess at the answers!

[1] In practice, more than three parallels would be used. This would increase the validity of the conclusions, because permitting of a more accurate estimate of error to be obtained.

Statistical analysis shows that:

(1) The decrease from chalking is *not* significant.

(2) The increase from phosphating *is* (highly) significant.

(3) The further increase from adding chalk to phosphate is *not* significant, and is mainly due to the effect of the phosphate.

In other words, chalk has done no harm; and phosphate has done a lot of good, not much helped by adding chalk, too.

The farmer can be told of this, with the added knowledge that the chance of his not getting benefit from phosphate is about 25 to 1 against, and that it is inadvisable to add chalk (or "lime") as well.

The form of the experiments which you have been doing and discussing in and since Chapter VII is called factorial, because such experiments allow for the interplay or interaction of factors. Conclusions and advice can be *soundly* based upon statistical analysis of *any* data that can be put into factorial form. That is a great improvement over the guesses which we should have to make without statistical analysis. In the long run, half our guesses are wrong, and the "expert's" guess is as likely to be wrong as the beginner's!

CHAPTER IX

USING YOUR NEW KNOWLEDGE

You may like to go a little farther than just making bacterial growth visible. Having once got the bacteria to grow (to give yourself confidence in the method of Chapter VIII), you may give an actual useful application to the method by finding out which soils allow the bacterial growth to appear, and which soils do not give any growth of bacteria under otherwise the same conditions. You will then be performing a bacteriological analysis of the soils, by testing them for presence or absence of these particular bacteria. When I inform you that "these particular bacteria" are the free-living, nitrogen-fixing species *Azotobacter chroöcoccum*, which are very important in helping to maintain soil fertility, you will see that after a little practice you will be able to do a quite informative little piece of research in biological survey of soil or water, if you wish.

Suppose that in the trials you have just made, growth occurs on all the pots that have had carbohydrate: this result implies that the bacteria have in the soil all their requirements for luxuriant growth, except carbohydrate. In other words, the soil is not notably deficient in lime, potash, or phosphate. The experiment cannot be a test for deficiency in nitrogen, because *Azotobacter* is able to gather its own nitrogen from the air, if it is supplied with every other need.

In the foregoing form, the experiment cannot be a true test for potash deficiency, since you have added potash to all pots that receive phosphate. If the *Azotobacter* grows on the phosphated pots, but not on those which do not receive phosphate, there is no possible means of distin-

guishing whether the response is to phosphate or to potash, since both are present.

To test for potash deficiency, you will need to add phosphate without potash. This may be done by adding potash in a salt such as the sulphate, which contains no phosphate, and also using the sodium phosphates corresponding to the potassium phosphates already mentioned. You will be testing the effect of a fourth factor. Unless for a " phosphate " test you *substitute* a " potash " test, you will therefore have to double the number of pots previously used.

Scheme for Analysis of a Soil for Potash, Phosphate, and Lime Deficiency.—Proceed as aforesaid, but using disodium phosphate and sodium dihydrogen phosphate in place of the corresponding potassium salts. However, since the sodium salts normally contain water of crystallization which considerably reduces the amount of phosphate in a given weight of salts, specify when ordering that the sodium phosphates must be anhydrous (without water).

Divide the original soil into two portions, after sieving. To one add nothing, to the other add mannitol or starch. Divide each into two, and add a little chalk to one part of each. You will then have the four lots C, M, CL, ML. Make a 1 in 1000 solution of potassium sulphate.

Taking C as our example, make from it four separate pastes in pots, moistening with water (CO); potassium sulphate solution (CK, K standing for potash); sodium phosphate solution (CP); a mixture of equal parts of phosphate solution and potassium sulphate solution (CKP).

Similarly with the other three prepared soil samples. You will then have sixteen pots; better, two or three times as many, for you should have replicated every treatment two or three times.

By inspection after the pastes have been in a warm place for a few days (as before) you will be able to say with some

probability whether the soil you have tested is deficient in available potash, phosphate, or lime. If *Azotobacter* will not grow in presence of the carbohydrate you have supplied to the M series, the soil is lacking in something. If growth occurs only after addition of potash, it is potash that is lacking: and so on. For purposes of this analysis the C series is not really necessary, but it provides a useful check. As a rule, growth will not often occur in any treatment of the C series, because the production of bacterial growth abundant enough to be visible to the naked eye usually does not occur unless there is a more ample supply of carbohydrate than is normally present in the soil.

But, given all these conditions, *Azotobacter* cannot grow if it is absent from the soil. We must distinguish two conditions. If you are to apply this test with a view of determining the requirements of soil for phosphate, potash, and lime, it would be quite fair to add some *Azotobacter* to the soil first, in order to ensure the presence of *Azotobacter*, since without those bacteria the analysis will fail. On the other hand, if you are testing for the presence or absence of *Azotobacter* in a series of soils—that is, in order to make a survey of *Azotobacter* distribution—the rather elaborate series of treatments that I have suggested becomes unnecessary: all that one has to do to test for presence or absence of *Azotobacter* is to supply all its probable requirements. It would be unsafe to rely upon the soil to supply everything. What you would do, to detect the presence or absence of *Azotobacter* in a soil, would be to add mannitol, phosphate, potash, chalk, and moisture and put up two or three pots of that mixture of nutrients and soil. You would in fact need only to make two or three pots of mixture MLP (p. 92), using the mixed solution of potassium phosphates. This may seem so simple that you may wonder why I did not mention it earlier. You will, however, see that it would have required practically as much explanation, for the MLP mixture is fairly complicated: I have preferred

to lead up to it by degrees via the simpler mixtures CL, ML, and CP, MP; and I also thought that you would be better able to understand the rationale of the MLP mixture if you had first tried to observe the effect of the simpler mixtures.

So much for the test for *Azotobacter*: the MLP mixture is all that is wanted, and a number of soils can be rationally tested by its aid without using more than three replicate pots of one soil.

If, however, you require to use *Azotobacter* as a tool in testing for the plant nutrients phosphate and potash and lime, you must set up all of the sixteen treatments mentioned on p. 93 (or as many of them as are needed to test for the nutrients you wish to search for), and you must make two or three replicates—32 or 48 pots—for each soil. Further, you must introduce *Azotobacter*, unless a preliminary "MLP" test shows it to be already present.

Obtaining the Microbe "Pure."—There are two ways of getting *Azotobacter chroöcoccum* in order to introduce it to your little pots of soil. One way is to buy a culture and suspend the live bacterial cells in the water or solution which you are going to use for making the soil pastes. The other way is to grow some *Azotobacter* for yourself. This latter is the more fun.

One method of growing *Azotobacter* is already before you. By using the MLP mixture on a sufficient number of soils you can obtain a surface growth of *Azotobacter* on at least one of the soil pastes. This growth can be scraped off and used as if it were a pure culture. It will not be pure, but will probably be sufficiently pure for the purpose.

A neater method is to grow *Azotobacter* on a liquid medium.

Add to some tap water one five-hundredth part of its weight of a mixture of two parts of dipotassium phosphate and one part of potassium acid phosphate, also a hundredth

of its weight of mannitol (starch is not very suitable here) and a very small pinch of ordinary salt. This mixture will probably contain all the nutrient requirements of *Azotobacter*, and will be sufficiently neutral, but if your tap water is of moorland origin add a pinch of calcium carbonate as well. You need not sterilize anything. Shake well, and pour the mixture into a conical flask or a wide-mouthed bottle (not very tall) so that the bottom is covered to the depth of about three-quarters of an inch. Add either a pinch of soil or a tablespoonful or more of pond water, and shake well. Cover or stopper loosely, and leave undisturbed in a warm place for several days. By the middle of the second or third day you may hope to see the surface of the water covered with a thin opalescent film, which becomes thicker and more opaque and may even become wrinkled in a few more days. This film is mostly composed of *Azotobacter* cells: by pouring off the water it may be obtained fairly clean, especially if pond water has been used as the inoculum. You may then shake up minute, almost invisible, bits of the film with your various solutions, or better still, half fill the flask or bottle with a solution of one part of common salt in two hundred parts of water, and use small volumes of the resulting bacterial suspension to "inoculate" the phosphate and potash solutions just before you make the soil pastes. (The common salt added to the water in the flask prevents the *Azotobacter* cells from being disintegrated by pure water as a result of what is called *plasmolysis*.)

It is simple to prepare a number of flasks inoculated with different soils, or with water from different ponds, canals, and so on. Success is practically certain sooner or later, and with luck you will have *Azotobacter* growing in several flasks at once.

Two tips: Soil from a highly fertile garden or from a field known not to have acid soil is most likely to yield *Azotobacter*. Almost any naturally slightly dirty watercourse or

sheet of water will yield *Azotobacter*, but pure spring water and very clear river waters often will not. The very clean waters are free from carbohydrate materals—mainly plant debris—and so do not supply enough energy material for *Azotobacter* to live in them.

Some Theory.—You may well ask, why does *Azotobacter* grow like this on the surface of soil paste or a solution? Firstly, only aerobic (oxygen-loving) organisms will develop at the surfaces of nutrient media exposed to the air. Secondly, no source of nitrogen has been added. Hence only an aerobic nitrogen-fixing organism can grow; that is, one which requires plenty of oxygen and is not dependent upon combined nitrogen. Practically the only organism that fulfils these two requirements is *Azotobacter chroöcoccum*.[1]

The " secret " of the rapid growth of nearly pure cultures of *Azotobacter chroöcoccum* on the soil pastes and liquid media is, then, an adaptation of the media to the special requirements and rather peculiar properties of the organism. The addition of carbohydrate such as mannitol greatly favours the *Azotobacter*. The numerous kinds of possible competitors in the soil, able and ready to utilize the carbohydrate for their own growth, have their growth rapidly brought to a standstill for lack of combined nitrogen: the small quantity originally present is quickly used up.

If the impure surface cultures were kept a few weeks, the other bacteria would begin to attack the *Azotobacter* and would use the nitrogenous compounds elaborated by that

[1] The nodule bacteria of leguminous plants are aerobic, but can fix nitrogen only (so far as is known) in association with leguminous plants. In one sample of water from a Norfolk drainage-ditch I have met another species of *Azotobacter*—*A. agilis*—but that was mixed with a large proportion of *A. chroöcoccum* cells. To grow these two species of *Azotobacter* I initially used the method just described. The film that grew on the surface of the liquid medium could not be distinguished by the naked eye from a film containing only *A. chroococcum*. There is, in soil, another free-living nitrogen-fixing bacterium besides *Azotobacter*, but it is anaerobic (disliking free oxygen).

organism. A more or less amicable balance would be maintained as long as the carbohydrate lasted. Once the carbohydrate had been used by the *Azotobacter* and by its competitors profiting from the nitrogen fixed by *Azotobacter*, a third stage would set in, but these later stages do not concern us: it is only the first—the outgrowth of *Azotobacter*—that concerns us in this chapter.

Summary.—A sort of summary seems to be called for. The following are the main points of this and the preceding chapter, not necessarily given in the order in which they occur in the text:

Azotobacter chroöcoccum is an aerobic bacterium able to fix nitrogen while living free in soil or water. For growth and nitrogen-fixation it requires a carbohydrate as a source of energy; mannitol is perhaps the best (from our point of view, at least). It also requires moisture and phosphate, and potash, and calcium in soluble form, in which respects it resembles higher plants. It also requires a neutral reaction, in this resembling many higher plants. Like other bacteria and fungi, it does not, however, require light. It grows more rapidly in moderate warmth than in the cool.

Given all these substances and desirable conditions, a profuse growth of *Azotobacter* can be expected to be visible on the surface of moist soil or a nutrient solution, provided the organism was originally present.

The emergence of this growth can be used either as a test for the presence of *Azotobacter* in a soil or water sample, or as an indication that no nutrient deficiency exists. To perform the experiment for the first purpose is to perform a survey of *Azotobacter*, while the performance of the experiment for the second purpose is essentially making a qualitative analysis of soil for the principal plant nutrients other than nitrogen.

For the second purpose it is legitimate to add *Azotobacter* cells from a pure culture.

Azotobacter is widely distributed in fertile well-manured soils that are not acid, and also in many natural and semi-natural waters. You are invited to check this statement for yourself by the means I have suggested above. It may possibly give purpose to your next holiday.

CHAPTER X

SOME OTHER EXPERIMENTS

(*a*) IN LIQUID MEDIA

IF you have made the mud-pie medium and the liquid culture medium for *Azotobacter*, you will be able to follow the directions in this chapter without my explaining at length the necessity for each ingredient and mode of working.

Azotobacter is a bacterium. Here are a few experiments on the culture of yeasts from natural sources, the principles underlying the cultivation being similar to those underlying the liquid culture of *Azotobacter*, except that combined nitrogen has to be supplied.

Have four large flasks, able to contain about 1,500 millilitres, and eight small flasks, able to contain about 500 millilitres.

(*a*) Make up the following solution [1]:

Distilled or rain water	1 litre
Ammonium sulphate	1·0 grams
Sodium nitrate	1·0 ,,
Dipotassium phosphate	0·2 ,,
Potassium dihydrogen phosphate	0·2 ,,
Calcium chloride anhydrous	0·1 ,,
Magnesium sulphate hydrated ($MgSO_4.7H_2O$)	0·2 ,,

[1] You may have, or should obtain, a balance capable of weighing correctly to say a decigram (0·1 gm.) with forceps and metric weights from 50 or 100 gm. down to 0·1 gm. at least. (Sets usually include smaller weights which will not be called for in these experiments, and will in any case be of no use to you unless your balance is sensitive to less than 0·1 gm.)

Instead of getting for a few shillings fractional weights less than 1 gm. and the forceps needed to handle them, you may like to spend about 15*s.* and get a chain attachment for the balance. This is an immense convenience, so long as you remember to bring it back to 0 after use, or at least see that it is at 0 before beginning to weigh.

(*b*) Shake well, allow to stand a couple of hours, and shake again. Divide into two equal lots.

(*c*) To one lot add nothing (A); in the other (AS) dissolve cane sugar, 15 grams.

(*d*) Prepare some boiled water, which need not be cooled.

(*e*) Divide each lot of culture medium (AS: with; and A: without, sugar) into four equal lots, in the small flasks. Boil two lots of each for three minutes. Label A, AB (B for boiled), AS, ASB. Make up the four boiled solutions to the same volume as the unboiled, by adding boiled water (this operation keeps the concentration of salts, and of sugar, the same in corresponding flasks). You will then have about 125 millilitres in flasks of much greater capacity, and the liquid will be in a thin layer on the bottom.

(*f*) To one of *each* pair of flasks containing similar solutions add any *one* of the following materials; to the other flask of each pair add nothing, keeping it as control; then plug all flasks with cotton-wool:

(1) A piece of baker's yeast about the size of half a hazel nut.

(2) A half or quarter of a stoned date (from boxed dates preferably).

(3) A grape, crushed.

(4) A whole grape.

(5) A raisin.

(6) A piece of fresh fig, with skin.

Manipulate the materials with a clean knife or spoon on a clean plate or tile. All of these, except the first, contain sugar. Note whether there is any difference in growth and odour-production between the culture solutions that already contain sugar and those that do not.

I say "add only one of the materials to each of four flasks," because you then have four treatments of each material and corresponding "controls." If you wish to test more than one kind of material you should make up a set of four flasks for each material. The control

(uninoculated) flasks will of course serve for any number of materials. But as it is better to have two controls than one, I suggest that for two inoculating materials you make up sixteen flasks by using double quantities, and put up two inoculations of every single treatment, and have two control flasks of each treatment. If you prefer it, have duplicate flasks of one treatment, and single controls. What I do not want you to do is to put a number of different inocula, each into fewer flasks than you have treatments. Each of your inocula should go into each culture medium. Otherwise, you will be unable to make any valid deduction regarding the effect of composition of medium upon growth, from such growth as you obtain.

Growth will appear (if inoculating materials bear or contain a viable yeast) as an opalescent film on the surface of the liquid, if the flasks are kept substantially undisturbed and in a warm place. For inspection, handle the flasks very carefully without agitating them. Do not remove the plugs except fleetingly to take a sniff, and even that is best done first after several days when growth is vigorous.

Modifications: (1) One or two flasks containing sugar, that have been inoculated, may be *very lightly* stoppered with a rubber stopper. After a few days remove the stopper. If it comes out with a " pop," gas—mainly carbon dioxide—has been formed by the action of the yeast on the sugar. This experiment is best done separately from the set of treatments. It would be safer to grease the stopper, so that it can slip out easily if the pressure gets too high, otherwise there is a risk of explosion of the flask. As a further precaution, use a strong bottle instead of a fragile flask, but in any case do *not* stopper firmly.

(2) Test for development of acidity with the Universal Indicator, adding it to control and to treated flasks after a few days. The unboiled control (uninoculated) flasks containing sugar may develop acid, however, owing to the activities of miscellaneous micro-organisms that may produce

a turbidity in the liquid with or without forming a film on the surface.

(3) Examine the surface films microscopically, if you have access to a microscope. You will probably find a nearly pure culture of yeast in the inoculated flasks, but not necessarily the same yeast in different flasks that have received the same kind of inoculum. There may be mixed yeasts on the fruit, while it may happen that only one species gets the upper hand in a given set of experimental conditions.

A different type of yeast can (probably) be induced to grow on a similar medium to which has been added at least 5 per cent. of common salt. The ordinary yeasts with which you have just experimented will probably not tolerate so much salt. You can test this by dropping a raisin, grape, etc., into a salty culture medium, or by transferring into the salty medium a little of the film that had developed in the previous experiment.

Salt-tolerant yeasts can only be expected to be present in quantity on such salty materials as sauerkraut, salted cucumbers, beans, and so on; therefore these and similar materials would be the most hopeful starting-points for making isolation-cultures of salt-tolerant microbes.

Prepare therefore the medium as before, containing 3 per cent. of sugar, and divide it into four flasks. To two of them add 5 per cent. of salt and to the other two add (say) 8 per cent. of salt. Since your present inoculum—unlike the grapes, etc.—will not be very rich in sugars, you may omit the medium without sugar. There will probably be no growth on that for lack of sufficient carbohydrate. The carbohydrates originally present in the vegetable will have largely disappeared during an acid-forming fermentation, similar to those discussed in Chapters V and XIII. Boiling the culture medium is desirable (as always), but is not essential, since the salt will act as a check to all but salt-tolerant organisms—it will, in fact, be exerting a partial antiseptic effect.

Into one flask of 5 per cent. and one flask of 8 per cent. salt medium drop a little piece of sauerkraut or any other salted pickle you can get. Retain the other two flasks as controls, and, as before, watch for the formation of a micro-organic film on the surface of the inoculated flasks kept in the warm.

You may also try to get salt-tolerant organisms from soil, but the chances against success are rather high. Salted fish and raw salted hide usually bear highly-tolerant bacteria, but I cannot promise you successful growth by this technique from those sources, as the very salt-tolerant micro-organisms are rather exacting in their requirements: an 8 per cent. solution of salt may, in fact, not be strong enough for them ![1]

All these experiments are examples of the use of what is called an elective culture-method, which means a method specially adapted to encourage the growth of one kind of microbe and is unkindly towards ordinary microbes; the balance is thus tipped heavily towards the supremacy of one kind of organism.

(*b*) On solid media

The next experiment will be the last one before a great deal of descriptive matter, for without apparatus of a specialized kind you will not be able to perform many more experiments.

So far we have used liquid media, excepting only the special case of the soil paste. You may now like to make your first solid medium; it differs in only one important aspect from the liquid media. To the solid media you add all the kinds of nutrients which (I hope) you have come to regard as usual—namely, salts and a source of energy—and

[1] For references to the literature on microbes tolerant of very high concentrations of salt, see Hugh Nicol, *Food Manufacture*, 1937, **12**, 111 (No. 4, April) [8562[a]].

also, of course, water, but the medium will be made solid by the addition of gelatine.

For bacteriological work, about 10 per cent. of gelatine is required in the final medium. Steep 20 or 50 grams of clear gelatine overnight in 200 or 500 grams of the undermentioned solution, and gently melt the lot (without pouring off any excess liquid). In hot weather use up to half as much gelatine again. As I plan for you to use the gelatine medium to grow small numbers of added bacteria, you cannot omit to boil it to kill the numerous microbes already present. Though it impairs the jellying strength of the gelatine, the boiling is unavoidable in bacteriological work (but is quite unnecessary in the ordinary way of using glues and gelatine [1]).

A formula for the culture solution to which the gelatine is to be added, is conveniently the same as the one on p. 104. You will, however, require to add about 10 grams of cane-sugar, or glucose, as energy material. (I suggest making a litre on account of the difficulty of weighing out very small quantities of salts. A fifth or half of a litre will be ample for a simple preliminary experiment.) Boil the whole litre of solution, shake, pour out what you need for the gelatine and plug with cotton-wool the vessel that contains the remainder. You will grow bacteria plentifully if you add a saltspoonful of "Lemco" or other meat extract before boiling. If you cannot obtain the chemicals, it may be

[1] Being a protein, gelatine contains nitrogen in a complex combined form. Like many other proteins, it is readily hydrolysed—that is, split up into simpler though still complex compounds, by the prolonged action of hot water. The products of hydrolysis of gelatine are not adhesive and do not "jell." Therefore, whenever you have occasion to use gelatine or glue, whether for carpentry, the arts, or for culinary purposes, do not boil the substance in water "until it is dissolved," as so many recipes say. The proper way is to steep the gelatine or glue some time—say overnight—in excess of cold water. If the material is intended for use as an adhesive, pour off the unabsorbed water. Gently heat the jelly-like material, alone—best in a double vessel—until it melts. It will then be ready for use with its properties as little impaired as possible.

useful to remember that almost any weak soup or broth will enable *some* microbial growth to take place, especially if a little sugar is added.

Have ready a few small saucers, not cracked or chipped, with some other saucers or plates large enough to cover the first. Boil them all for ten minutes, then pour off the water, and allow to dry without handling. Alternatively, bake them. In the later manipulation, handle the saucer by the edges with clean hands.

Having prepared the hot jelly, pour it to a depth of about a quarter of an inch into each of the small saucers, *cover* them, and allow to cool, in a place free from draughts.

Now sprinkle a minute trace of powdered soil over the jelly—a mere almost invisible dusting will suffice, and keep the saucers at room temperature (in summer), or in a slightly warm place (in winter). Watch for the development of colonies of bacteria around the soil particles, on the " plates " of gelatine medium.

Another way is to shake a little soil in water as you did in Chapter VII, and to add a *trace* of the resulting bacterial suspension to the tepid jelly, and let it set.[1]

If you choose a colony which is not touching any other, you can assume (as a first approximation) that the colony is a pure one. " Pure " means that it is composed of only one kind of bacteria or fungus. If you have already a sterile plate of your gelatine medium, you can touch the selected colony with a wire, sterilized by passing it through

[1] You will probably find that your gelatine goes liquid in places when it has not been heated enough to melt it. This liquefaction is due to gelatine-digesting enzymes (see p. 73) possessed by many bacteria. Such liquefying spots tend to spread and to spoil the integrity of the colonies on firm gelatine.

The liquefaction is itself interesting. The liquefied gelatine at a spot where the liquefaction has not proceeded far will, very likely, have a pure culture of one of the " liquefying " bacteria in it. Dip a sterile wire into such a spot. If with this wire you stab a tube of gelatine medium, solidified while the tube is upright, you can watch the process and development of liquefaction.

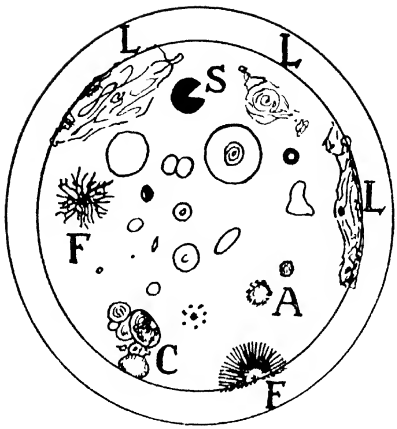


Fig. 7.—Showing semi-diagrammatically the sort of thing that may be expected on a saucer of gelatine medium that has been inoculated with a very little of a highly diluted suspension of soil. Numerous microbial colonies develop on the plate.

A: two actinomycete colonies

F: colonies of two species of fungi.

The other colonies are all of bacteria. Most of the colonies are shown discrete, *i.e.* not touching any other, therefore being suitable for picking-off as the basis for presumably pure cultures. At C several confluent colonies are shown, and there is elsewhere a pair of confluent colonies. S indicates a rare form of colony: said to be "sectored". L indicates colonies- or rather, areas of influence- of bacteria able to liquefy gelatine. In practice these will not usually respect the areas occupied by non-liquefying organisms, nor will the fungi refrain from spreading.

For the sake of clarity, the fungi are here shown at a stage rather earlier than that of the bacterial colonies. By the time the bacterial colonies have reached the sizes indicated, the fungi will usually have overspread the surface of the medium to a considerable extent.

Colonies may be transparent or opaque, whitish or coloured. Note the radial and concentric types of colonial growth—consequences of growth from a centre. Spreading and arborescent (branched) colonies are also common.

a gas-flame, then streak the infected tip over the surface of the new medium. You will thus obtain a pure culture of the selected microbe. If you streak the wire over part of the sloped surface of the same or another solid medium in a plugged tube, in which the medium has been allowed to set while the tube was nearly horizontal, you can not only watch the development of the bacteria as an elongated colony along the streak, but can preserve your pure culture for a time, until the medium dries up, or until you transfer again. If you dip the infected wire into a liquid medium or series of liquid media, you can easily study some effect of the selected bacteria: acid production on various sugars, ammonia production from peptone, and so on. You have already used a liquid medium for some purposes, such as watching for film-formation with *Azotobacter* and yeasts.

Plating, therefore, offers a method of separating mixed bacteria, provided a dilution is made that is sufficiently high to give a fair chance of the formation of discrete colonies. You may like to try to separate a simple mixture of distinctive and differently coloured bacteria. You can make a mixture of such bacteria after picking some off your own plates.

To obtain a substantially pure culture such as is represented by the majority of the colonies in Fig. 8, dip a flamed, but cooled, wire into one of the discrete colonies from such a plate as is shown in Fig. 7, and whisk the infected wire around in one of three or four plugged test-tubes which are about a third filled with tepid gelatine medium (tepid, so as to have the medium liquid but not to kill the bacteria); then pour not more than two drops of the bacterial suspension thus made into another tube; swirl the contents of the second tube (without introducing the infected wire), and pour two drops of its contents into a third tube, and repeat with a fourth if desired. Do not touch the rims of the test-tubes. Pour the still liquid contents of each of the tubes into a saucer, cover with another sterile

saucer, and keep the " plates " of medium out of draughts. After the gelatine has set, it is a good plan to invert each pair of saucers, so as to have the gelatine surface " looking down." The first and second " plate " will probably give rise to a very dense growth (too many colonies), but the

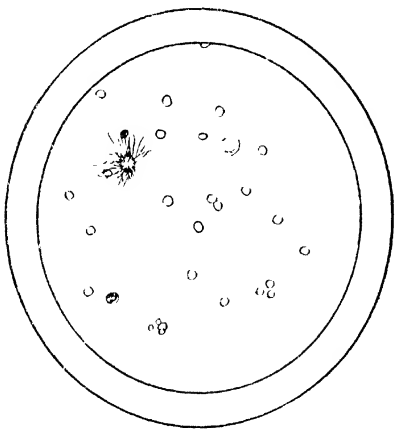


Fig. 8.—A nearly pure culture on a saucer " plate " of gelatine medium. This shows the sort of thing a beginner may expect to get if he tries to obtain a pure culture without having special laboratory facilities. There are two or three colonies of obvious bacterial contaminants, and also a fungus contaminant. (See Chapter XII for explanation of " contaminant ")

third and fourth may be more or less as shown. Should your empty (sterile) saucer be larger or smaller than the one with medium in it ?

You may like to try to prepare " plates " from tubes inoculated with a drop or two of decomposed disinfectant solution from one of the middle vessels of the experiment in Chapter VII (Fig. 6). Use a sterile spoon to obtain the in-

oculum. You may try to see how many kinds of bacterial colonies can be recognized, or to prepare pure cultures of one or two kinds. You may use the ordinary gelatine medium, or one to which a trace of disinfectant has been added. (You will lose some disinfectant while boiling the medium.)

A very simple and instructive variation is to add to the gelatine medium, before boiling, enough precipitated chalk to render the medium just turbid. After adding a drop or two of soil suspension to a tube of medium, pour it on to a dish of clear glass, cover, and watch for the development of clear zones around certain colonies: these will be colonies of bacteria that have produced enough acid to dissolve the chalk, the acid having diffused through a zone extending well beyond the site of the colony proper. Quite an impressive exhibit is formed by a few colonies of a pure culture of such bacteria growing on a glass "plate" of chalked medium.

CHAPTER XI

COUNTING THE MILLIONS

" *Thirty times three makes ninety. I wonder if anyone's counting?* "
" Through the Looking-glass."

By using an extension of the methods just described, it is possible to make an estimate of the numbers of viabel bacteria in a sample of soil or other material. " Viable " means alive and capable of growing. The estimate of numbers is rightly or wrongly called a " count." Plating methods cannot be used to determine the total numbers of bacteria, including dead ones, in a sample of soil; a microscope must be used to do that.

To adapt what you have just learnt to the " counting " of the number of viable bacteria in a sample of soil, all that is necessary in principle is some simple arithmetic. If one gram (saltspoonful) of the soil is shaken up with, say, 250,000 grams of water (so as to give a countable number of colonies on a plate), and one gram of that dilute suspension of bacteria is added to a little tepid gelatine medium, which is then allowed to set: if, say, forty colonies develop on the resulting plate, there presumably are in every gram of that soil about 40 × 250,000 viable bacteria (10,000,000 per gram). It is as easy as that !

There are, however, some practical points about the process of " counting " bacteria in this way which make it hard for me to recommend you to do it except for fun. You may like to count fungal and bacterial colonies separately; actinomycetes may also appear. Bacteria able to liquefy gelatine jelly are likely to be a nuisance; that is why in laboratory work we use agar, and not gelatine, for soil " counts." To do everything accurately requires more

apparatus than I can expect you to have; it also demands a high degree of skill in no-bacteriology, without which you would never be confident that your colonies arise from microbes that really were in the sample you used.

Since 250,000 grams of water is about five hundredweights, you will need to adopt some dilution dodge in order to have a manageable amount of liquid; say, a modification of that given in Chapter VII, but having successive dilutions in the ratio of at least 10 to 1, instead of 2 to 1. If you shake 10 grams of soil in a litre of water, you can get a dilution of 1 in 100,000 with three and one in a million with four cups.

In principle, however, the method outlined on p. 115 is the same as that used for "counts" of soil bacteria by plating. It depends on the assumption that the bacteria having been well dispersed (separated from each other) by shaking, each bacterium that is capable of reproducing and living in the chosen medium will multiply on the site in which it is fixed by the soil jelly, and will go on multiplying until it assumes the dimensions of a colony visible to the naked eye. A simple calculation: average number of colonies per plate multiplied by dilution (*i.e.* relation that the volume of suspension of soil bacteria added to each plate bears to one gram of soil) gives the number of bacteria per gram of soil. The name "count" is really a misnomer, for while one actually counts the colonies on the plate, one cannot count the number of bacteria in a gram of soil, and the "count per gram of soil" is in fact an estimation.

Even the best plating methods give numbers that show not much more than 50,000,000 bacteria and actinomycetes per gram of ordinary soil. By "ordinary," I mean, that the soil has not recently been manured or had decomposable matter (energy material) added to it. As you learnt very early in this book, the total number of microbes in ordinary soil is about fifty times as large. The discrepancy is due largely to the fact that no one medium will allow all the

bacteria to grow. The plate-count is suitable for comparative purposes only.

Whatever you do, you will not (I hope) do a "count" with just *one* plate, or even two. You will replicate at least three times; that is, have at least three parallel plates, using the same amount of soil suspension, and having everything else the same. Your results may be expected to confirm each other. If the actual counts of colonies are widely different, something may have gone wrong: you may have had too many contaminants, or you may have added the soil inoculum twice to one plate.

Suppose your actual counts of every colony (bacteria, actinomycetes, and fungi) on each of three plates are 27, 30, 36—giving an average of 31; and suppose that on a parallel set made at the same time with the same soil you get 10, 29, and 54—also giving an average of 31. Can you rely on these results being true for your conditions? You are in effect taking random samples of the soil microbial population. You should not be over-pleased if only three parallel sets of counts agree closely or even coincide; that may be due to chance. On the other hand, if any set of parallels shows wide variations (we say, has a considerable *variance*) it suggests that something is wrong. The number of colonies on the second set of plates has a wide "scatter." Mathematical analysis shows that its degree of variance is more than what can reasonably be expected from chance; therefore the second set does not support the first, and the results of the second set must be rejected. You are consequently left with only one set, which has no real support for the validity of any conclusions you may infer from it. You will see that when working with random samples, no valid conclusion can be drawn from averages alone, even if they appear to support one another. I may add that the amount of variance, and consequently the chance that results are trustworthy, is calculable. For example, thanks to statistical analysis, it is possible to say of accurately

performed experiments (such as laboratory counts of colonies), whether the odds against a given result being due to mere chance are greater than 20 to 1.

In the past, and, indeed until the last fifteen years or so, action about microbes, fertilizers, motor-cars, and everything else had to be based on consideration of mere averages. These, you have learnt, may be highly misleading (because giving a false sense of correctness); furthermore, averages cannot " tell " us whether they are valid or not.

Averages are for that reason one of the stand-bys of the plausible dealer in figures. Many people have been induced to take up farming in new countries with the assurance that " the average rainfall is 30 inches." It is possible to farm successfully in Britain with an average rainfall of 30 inches, because the actual rainfall never varies much from that amount. But those who believe a real-estate man's " average of 30 inches " would be saved much heartbreak, and possibly ruin, if they knew that his figure was an average of 50, 19, and 21 inches. It does not require a statistical analysis of variance to see that the crop would probably have failed twice in three years. In general, it seems legitimate to enquire into anyone's motives, if they present a mere average as a basis for conclusions, or attempt to justify a comparison by basing it upon two single figures.

Scientists would not do so if it were only a matter of a couple of plate-counts of bacteria. In public matters, the need for statement of the original figures, or of the conditions, or of an estimate of error or variance, or all four, is still more necessary.

The new technique of mathematical analysis is clearly a gain. If action is taken on the basis of results of suitably planned experiments based on truly *random* sampling, that action will be wrong only once in twenty or some determinable number of times. This is true whether the data are obtained from microbes, motor-cars, or anything else.

Upon direct microscopic examination of a sample of soil

which has been suitably stained to reveal the bacteria, it is found that the total number of bacteria in resting soil is at least as many as 2,000,000,000 per gram. It is necessary to use a high power of the microscope in order to see the bacteria, and as a result only a very minute fraction of a gram of soil can be explored at one time and have its bacteria counted. This fraction is about one-hundredth millionth of a gram. It is impossible by any means to weigh or measure such a small amount, so a dilution method

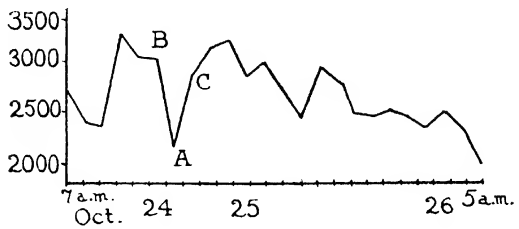


Fig. 9.--Fluctuations in total numbers of bacteria in an unmanured plot of soil at Rothamsted in 1934. The "counts" were made every two hours for 48 hours. The sampling of the soil is shown in Plate VIII. The numbers on the left of this diagram represent millions of bacteria per gram of soil.

is employed to begin with. The bacteria are actually counted by microscopic examination of a very thin film. In order to get over the difficulty of measuring the amount of soil that is visible in a microscope, a known weight of soil before dilution is mixed with a known volume of a suspension of indigo particles each about the same size as a bacterium. The number of particles of indigo is previously counted in an apparatus which is essentially the same as that used for counting red cells in blood. (The counting of blood cells or indigo particles can be performed easily; they are in suspension in a liquid uncontaminated by opaque irregular solid particles.)

Having been stained a bright red, the bacteria can be counted in a specially marked-off part of each circle of view (called a "field") that is revealed by the microscope. The contrastingly coloured indigo particles can also be counted in the same field. Things are usually adjusted so that the number of indigo particles is about the same as the number of bacteria. The ratio of bacteria to indigo is ascertained from examination of a large number of "fields." Simple arithmetic, as before, then gives the total number of bacteria per gram of soil, since both the extent of dilution and the number of indigo particles that have been added are known. This method of estimating bacterial numbers in soil is conveniently known as the ratio method.

Results obtained by the ratio method are submitted to statistical analysis. We therefore know that many of the fluctuations in bacterial numbers shown in Fig. 9 (p. 119) considerably exceed the calculable errors. They are therefore real.

CHAPTER XII

NO-BACTERIOLOGY

THE growing of microbes is not generally the most difficult part of the work in a microbiological laboratory. To grow bacteria, yeasts, and fungi is very easy. If the experiments have not convinced you to the correctness of this claim, you will have noticed for yourself that mould-fungi grow spontaneously on damp bread and other solid pabula; and one need say nothing of the ease with which milk can be induced to become sour in summer!

The argument may be turned the other way, so as to suggest to you, if you have not tried to do any of the experiments, that most of them are easy after all. The chief difficulties you will have with them will be to obtain the necessary chemicals (not so very difficult, this), while in some of the experiments it will be bothersome to do the measuring, and also to prevent unwanted microbes from getting access during the preparation of your cultures.

The only one of these difficulties that faces the professional microbiologist is the last. The requisite chemicals are at hand, and can be obtained as readily as the housewife fetches edible stores from the pantry. Measuring is not a trouble to the professional, for he or she has been trained to do it, and has most likely forgotten his or her 'prentice essays. But the keeping-out of unwanted microbes is an ever-present difficulty. Unwanted microbes growing on culture media are called "contaminants"; they come in from room, air, and dust; sometimes from unsterile apparatus, or from the person or clothing of the experimenter.

It is not too much to say that the skill of a bacteriologist can be gauged best from the degree of consistent success he or she attains in keeping contaminants to a minimum. That

is why among the first experiments described for you, as a beginner, to do for yourself, I have put those (elective) methods that ensure good growth of some desired species in the presence of all likely competitors. Such elective methods are therefore, if I may say it, practically fool-proof, and the one given on page 81 is wholly so. Incidentally, it may be said that this approach is not one adopted by the textbooks; they usually start with some form of the relatively difficult methods like those of the last chapter, and reserve the elective methods for the advanced student.

So far as microbes are concerned, the whole aim of the surgeon is to keep them out. The microbiologist has to grow some microbes, but not others, hence he is not free to do all that the surgeon can. I do not mean to suggest that the microbiologist's job is more difficult than the surgeon's—I mean only that it is different in kind. To speak in parables, the microbiologist has to separate the sheep from the goats, while the surgeon is a vegetarian and abolishes them all.

He does this by a combination of antiseptic and aseptic techniques, but the microbiologist's position is peculiar; and the methods the microbiologist adopts to keep out contaminants can best be called no-microbiology.

The phrase " aseptic technique " may be used instead, as a convenience, provided its limitations in microbiology are understood, but no suitable word has been invented to describe the state of some microbiological techniques. For example, if leguminous seedlings are grown in presence of nodule bacteria, all other bacteria being excluded, can the cultures of plant *plus* bacteria be called sterile? When some seedlings are grown as " controls " (*i.e.* for purposes of comparison) without any microbes whatever, are the cultures then " sterile "—while living plants are present? If we say the latter cultures are " aseptic," what do we call the former?

NO-BACTERIOLOGY

In your elective experiments you adopted methods that practically compel the right bacteria to grow. In advanced microbiological work, it is often necessary to grow some special kind or kinds of microbes on media on which other microbes will grow only too easily if they get the chance. The job of the bacteriologist (for example) is as much concerned with not growing any undesired bacteria as with growing those he wants. I don't think this has been put into words before. Not to grow bacteria might imply annihilating them by heat or disinfectants, or not encouraging any to grow at all (the surgeon's aim); but not growing some, while growing others, seems to me to want a word. Hence the title of this chapter.

I had intended to give a description of a complete bacteriological operation as done by an expert, and to include a full account of every precautionary move against the intrusion of contaminants. The description would have been a sort of model examination answer. There is so much to be said yet, that I renounce further discussion of no-bacteriological methods. But perhaps you are thinking that one of the sources of contamination could be removed by using filtered air, and that my model answer would have lost marks for not mentioning so obvious a precaution.

The filtration of air is invaluable in strictly aseptic work, such as that shown in Plate VII. Its value in no-bacteriology is not what it may seem. The conditioning of air implies a current, whereas no-bacteriology is best performed in absolutely still air. Also, it is a basic principle of scientific work that it is unnecessary to trouble about possible errors that are demonstrably smaller than those which cannot be avoided.

A million million microbes—more or less—are present in a cupful of soil received for examination (which includes sieving or grinding) in a laboratory. It does not seem to be wise or necessary to remove a few bacteria (perhaps a hundred) from every cubic metre or cubic yard of air going

into that laboratory. About how long would it take for a filtration plant to remove as many bacteria from the air as there are in one cupful of soil, if ten cubic metres of air (containing 1,000 bacteria) passed through the plant per second? (This is the only arithmetical question in the book; the answer is on p. 239.) Perhaps I caught *you* that time?

CHAPTER XIII

BAD TO BE GOOD[1]

IN laboratory practice, unwanted microbes may be inactivated by use of disinfectants and antiseptics, or by heat, or they may be removed by filtration through a material that has pores small enough to retain the microbes. The use of disinfectants or antiseptics implies the addition of some chemical, which, if it kills the unwanted microbes, will probably kill any other later introduced into or on the nutrient medium; hence, antiseptic methods are rarely suited to pure-culture study of microbes.

The use of heat is often admissible, but it is unsuited for materials which appreciably alter their composition on heating. The prime resource in the microbiological laboratory is to keep unwanted microbes out. One method of securing a medium that is sterile at the outset is to take, with all aseptic precautions, a piece of tissue from a healthy animal: a piece of liver from an animal recently killed, or a fragment of a developing egg. Microbes may be filtered out of an originally contaminated liquid. The difficulty thereafter is, as I have said, to prevent microbes of every stray kind from gaining access to the sterile medium; elaborate precautions have to be adopted for this purpose.

However feasible such precautions may be in the laboratory, their translation on to a factory scale is a problem in itself. That is why the marketing of unheated apple juice

[1] This chapter is an expansion of an article which appeared under the same title in the issue for 22nd April, 1938, of *Food Industries Weekly* [8561/1], and is substantially reproduced by courtesy of the proprietors of *F.I.W.*, Messrs. Leonard Hill Limited, London, W.1.

that will " keep " has only recently been accomplished. " Keeping " implies the absence of change due to microbial and other activity. Not only is the flavour of apple juice impaired by heating to temperatures at which all microbes are killed, but the addition of more than a small amount of antiseptic is inadmissible for the same reason—apart from legal considerations.

Hence, fruit juice that is to be sold as " unheated " must be specially filtered to remove microbes inevitably present in it during the first stages of processing: and it must be filled into sterile bottles or cans, care being taken that microbes from the air or from the hands or clothing of the operators do not contaminate it anew.

It is not surprising that the application of " aseptic " methods on the factory scale to highly mutable products is a development of the last few years: it is a technical achievement of high order, made possible by modern methods. But don't think that our ancestors were therefore unacquainted with the preservation of fruit juices. The methods they used were just the opposite of aseptic, and they called the products wine and cider.

When Alexander was marching towards India, he left a land of wine for a region wherein the chief drink was milk preserved by fermentation. Asiatics were and often are too casual to keep milk sweet, and they still keep milk good by making it bad. So do Europeans; but Westerners have not acquired, or have outgrown, the taste for liquid fermented milk; they generally prefer their microbized milk to be solid, and they call it cheese.

Professor J. C. Drummond made a slip in his 1937 Cantor Lectures on " Historical Studies of English Diet and Nutrition." Introducing the subject of canning, he wrote:[1] " The opening of the nineteenth century saw the first successful attempt to preserve foods by other methods than the

[1] *Journal of the Royal Society of Arts*, 1938, **86**, 246–58 *et ante* [11537].

age-old use of salt and vinegar." Was not Noah a historical character?[1]

So, salt and vinegar are not the only preservatives of food. Why consider pickled cabbage (preserved with salt and vinegar) and omit to consider sauerkraut, preserved mainly by micro-organisms with a little salt? The preservative properties of vinegar are due to acetic acid, which at ordinary strengths discourages the growth of most micro-organisms, but the lactic acid produced by the micro-organisms of sauerkraut and silage is an equally effective preservative: to silage no salt is added. Excavations in Egypt have shown that the preservation of fresh forage in the form of silage was known in ancient times. Professor Drummond was referring only to human food, which silage is not. But the quality of silage has a bearing on human food, and I am concerned in the general question of microbes being used in the preservation or improvement of perishable foods of any kind.

Ham is not merely improved by bacteria, but is more truly a product of bacterial activity than is salted cod or a brined herring, for the purpose of salt in the merely salted or brined foodstuffs is to repress entirely the growth of microbes. Ham, on the other hand, owes its pink colour indirectly but certainly to the action of micro-organisms on saltpetre (potassium nitrate); this is "reduced" (as chemists say) from nitrate to nitrite by bacterial action, and the nitrite combines with red hæmoglobin of the meat to give that appetizing pink.

Cheese is so well known that a detailed discussion of it would be out of place. Someone has said that "cheese is milk grown-up"; the phrase is apt, in view of the numerous micro-organisms that have lived in cheese and have imparted a biological cachet to that product.

In this sense, cheeses, and fermented milks, are decidedly grown-up, for at every stage they contain an abundance of

[1] Genesis ix. 20–21, 24.

micro-organisms. It may be recalled that some cheeses are not considered to be good until they are nearly bad. The Limburger, of which part of the microbiological history is given on p. 168, is one. The more odoriferous cheeses are the seat of a prolonged and fluctuating struggle for microbial supremacy; first the microbes attack the lactose of the milk; next they assist in changing the casein to a semi-permanent form, in which it is resistant to attack by most external micro-organisms, until finally the casein is surrendered to an internal attack; at that stage, man, the macrobe, steps in and settles the question of supremacy as far as the cheese micro-organisms are concerned.

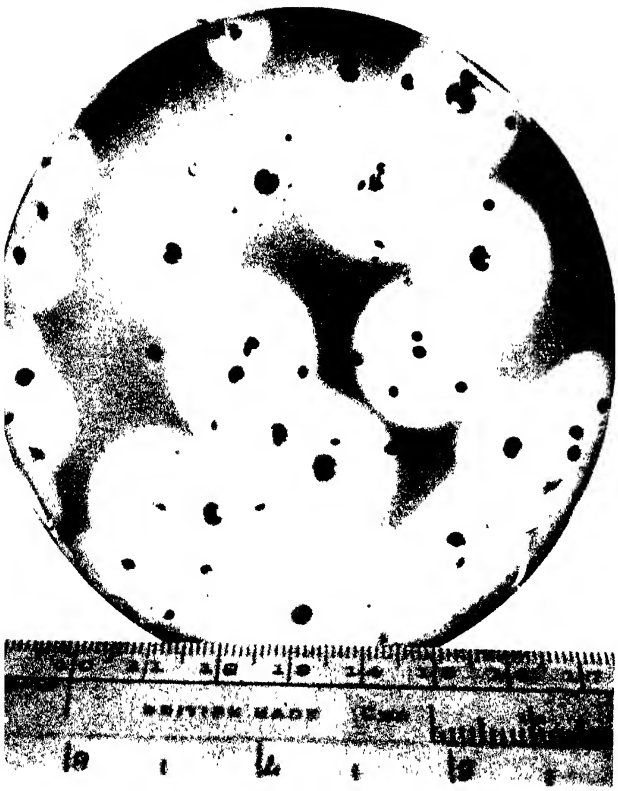
When cucumbers and similarly carbohydrate-rich materials are salted, the salt does not suppress microbial activity. It merely assures the predominance of those kinds of acid-forming microbes that can tolerate high concentrations of salt. This may seem to contradict what has been said about salt being used for the entire repression of the growth of microbes in salt fish.

The key to the apparent contradiction lies in the fact that fish and meat are poor in carbohydrates, so but little fermentation can go on, and that only while the material is fresh and moist. You will recall that in the curing of hams it is usual to add not only salt and saltpetre, but sugar or treacle as well, to serve as energy-material for the bacteria.

Silage can be made without adding anything to the green fodder that is to be fermented. This demands some skill, if a palatable silage is to be the result. With many materials —especially those rich in protein—it is a help to add some treacle (molasses); the microbes preferentially use this for the production of lactic acid, instead of breaking down the constituents of the fodder and possibly producing an inferior article.

In the making of silage the rôle of the microbes seems to be solely that of producers of acid, though it is not impossible

PLATE I



A partly-purified culture on an agar plate of an agar-digesting bacterium showing the circular zones of influence around each colony indicating the limits to which the agar softening enzyme has penetrated. The softening of the agar has not interfered with the growth of colonies of bacteria unable to soften the agar. This uncommon organism was obtained from a London suburban garden.

Acid-producing bacteria on a chalked medium show similar circular zones (cf. Chap. XI, p. 114)

PLATE II

Strains of the "miraculous bacillus" (from the Norfolk Broads Horsey New Cut), showing differences of intensity of colour when grown on several media sloped in test-tubes (page 195).

PLATE III

"Toadstools" (*Coprinus* species) growing spontaneously on chopped oat straw in a small flask. Such growth occasionally occurs during studies of the rotting-down of straw

(Photo H. G. Thornton.

A pure culture of a cellulose-decomposing bacterium attacking a fibre of filter-paper. This bacterium exists in two forms (coccus and vibrio), both of which are shown.

PLATE IV

Nodules each the home of millions of symbiotic nitrogen-fixing bacteria on a soya-bean root compared with a sixpence. The nodules on most other kinds of leguminous plants are very much smaller than these

PLATE V

PLATE VI (A)

Variation in effectiveness within one species of bacteria

Both pots of sand contain lucerne, sown on the same day, and supplied with all needful chemicals except combined nitrogen. The pot on the left has been inoculated with a moderately good strain of lucerne nodule bacteria, that on the right, with the best strain known to Rothamsted. The difference in growth of the lucerne is due to variation in the ability of the strains of bacteria as fixers of atmospheric nitrogen.

Several thousand cultures of the best strain are annually sold to farmers by Messrs. Allen & Hanburys Ltd., being periodically tested by Rothamsted to see that it maintains its qualities.

PLATE VI (B)

A longitudinal section of an active nodule on a broad bean, the root being to the extreme right and the tip of the nodule to the left.

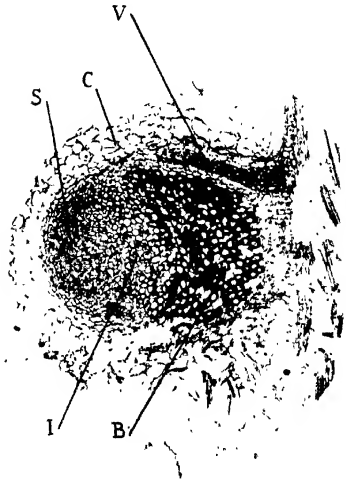
C outer coat (cortex)

S sterile and youngest growing tissue

I a recent growth of nodule tissue becoming infected from

B a congeries of nodule cells already largely filled with bacteria (stained and showing as black masses). It is supposed that fixation of nitrogen takes place in this region.

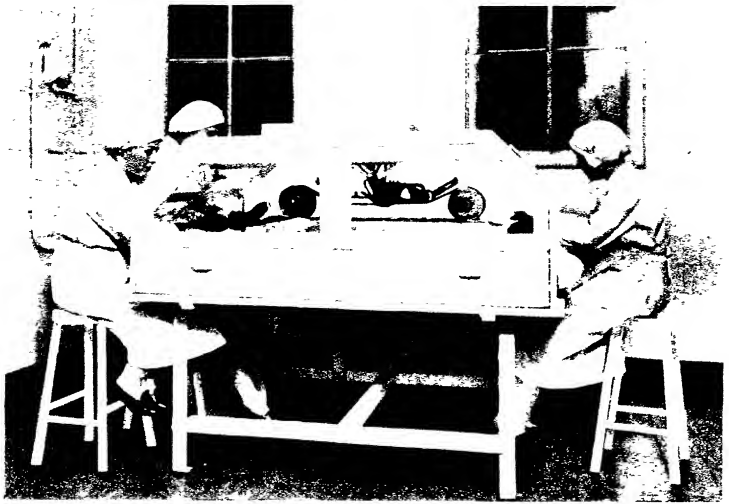
V a vascular strand, or tube, through which sugar passes from the plant towards the bacteria in the nodule.



(From the paper by Winifred E. Brenchley and H. G. Thornton, *Proc. Roy. Soc. Lond.*, 1925, *B*, 98, 373 [16900]. By courtesy of the authors and the Royal Society.) The actual size of the nodule is shown by the o at the side.

o

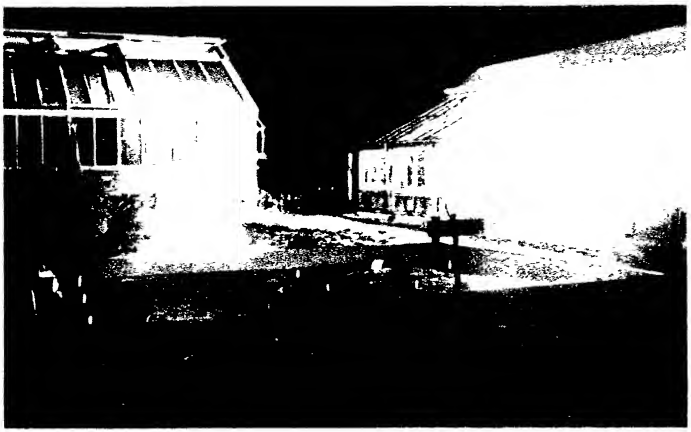
PLATE VII



(By courtesy of Messrs S. Maw, Son & Sons Ltd.)

Rigorous asepsis in a factory.—The manufacture of surgical dressings by special machinery. The machine is in a cabinet situated in a room built and used like an operating theatre and supplied with filtered air. Every pulley and other part of the machine is sterilised before use, the machine is then assembled under aseptic conditions. It is worked in an outward current of sterile air introduced from the centre of the cabinet.

PLATE VIII



Soil bacteriology done by night.—The "count plot" of soil at Rothamsted as it appeared when being sampled by night to give the results of Fig. 9, p. 119. Light was provided by hurricane lamps and by the illuminated greenhouses

that they may be producing a vitamin-like substance too. When the microbes of silage or sauerkraut or salted cucumbers have produced enough acid to act as a preservative, their own action is inhibited, as well as the action of most other microbes. It is not economically advisable to make silage by adding lactic acid in sufficient quantity at the start. A method of largely short-circuiting the fermentation microbes of silage has been worked out recently, and is called the A.I.V. process—from the initials of its inventor, Professor Artturi Ilmari Virtanen (accent on the *first* syllable of each name, please !).

The A.I.V. process consists of adding enough of a mixture of dilute mineral acids, such as hydrochloric and sulphuric acids (spirits of salts and oil of vitriol, respectively) to the green fodder to bring it to the same degree of acidity as good silage normally has (see p. 218). Little lactic acid is formed, and the losses of nutritive value are small: wasteful breakdown of carbohydrates and proteins is diminished, and the amounts of vitamins remain very high. Mineral acids are not poisonous unless they are strong enough to be corrosive, and those added to the silage are almost wholly neutralized by constituents of the fodder. About 300,000 tons of A.I.V. silage is produced and consumed annually in Finland alone.[1]

Some curious foodstuffs have been prepared in the attempt to preserve food by first making it bad through micro-organic action. That unstable substance—or mixture of substances—milk, has been the subject of many such empirical applications of microbiology. *Tarhó*, the Hungarian (or perhaps one should now write, Rumanian) fermented milk is an unappetizing-looking mass of the consistency and colour of liver. The old Bavarian *Herbstmilch* (autumn milk) was prepared in wooden boxes, was salted, and by its name sufficiently suggests that it was a

[1] See A. I. Virtanen, *Cattle Fodder and Human Nutrition*, Camb. Univ. Press, 1938. 7*s*. 6*d*.

substance prepared for storage until a season when fresh milk could no longer be obtained.

" Milk fizz " acquires a new significance when applied to the Central Asian kumiss, which contains not merely acid from souring of the milk-sugar, but also an appreciable quantity of alcohol, as well as enough carbon dioxide to make it froth. It is typically made in skins, which allow of easy mixing of the contents. Courses in dairy bacteriology have been brightened by the anecdote about the engaging habit of Central Asian nomads of giving a friendly punch or kick to the pendent milk-vessel of the neighbour upon whom they were calling or by whose tent they were passing. Recently, soya beans have been used to make fermented artificial milk, but since the beans are not very rich in carbohydrate, some sugar needs to be added to secure a satisfactory fermentation.[1]

Before wines are marketed they are usually deprived of most of the microbes which they contained during the primary fermentations. They may be looked upon as preserved fruit juice, but, as almost all their original carbohydrate has been used up by the microbes, the direct food value of wines is small. Spirits, as prepared by civilized methods, are practically sterile. Civilized people need not appeal to microbes for an excuse to consume wines and spirits, since neither class of fermented liquor can be said to be a source of micro-organisms having a nutritional value.

With native fermented drinks the case is different. Such native drinks are usually of the beer type, though what is said to be a peculiarly potent spirit is distilled in Siberia from fermented milk, and there are other, better-known, examples of native-made spirits. There is a story that some well-meaning temperance reformers induced the natives of

[1] For references see the article by Hugh Nicol, " Fermented Milks," *Food*, 1937, **6**, 196 (February, No. 65) [8561[a]]; and a correction in the same journal, **6**, 386 (July, No. 70).

a Mexican district to refrain from drinking the local beer (*pulque*), and the result was an outbreak of an unpleasant deficiency disease. It was found that the natives' solid diet was nominally unbalanced, but that yeasts in the crudely fermented drink provided the saving grace. Deprived of their *pulque*, the Mexicans manifested the effects of a deficient diet.

I cannot vouch for the absolute truth of this story, as I am relying upon memory of something I read in a journal so badly indexed that I have not been able to find the story again. However, in the issue for April, 1938, of *Economic Geography* [7585*a*], Mr. E. C. Lanks contributed a study of the Otomi Indians of Mexico, and he made the following remarks; largely supporting what I had earlier written:

" The beverage, *pulque*, which seems to loom so large in their cost of living, in many cases represents nearly a third of the total cost. The brutalizing effect of this mildly intoxicating drink has been much discussed. It is the fermented juice of the maguey [agave] plant, and averages a low content of alcohol. It must be drunk in large quantities, therefore, to be intoxicating. For the present it seems to be the main relief from a miserable life at subsistence level. It was formerly thought by the government that the lot of the Indian would be improved if the evil of *pulque* were removed. However, it has since been found by scientific investigation that the drink is probably a vital necessity for some, with their unbalanced subsistence diet, for it has been found to contain vitamins A, B, and E, proteins, fats, yeast, and amido [amino-] acids. It is doubtful if the race could have survived on their limited diet without this balancing food beverage."

A similar story of unwise interference has been told of Nauru, the British-owned phosphate island in the Pacific, but I have it on the authority (private communication) of Sir Albert F. Ellis that this allegation does not accord with the facts. (The recent history of Nauru may be said to

spring from the life and work of Sir Albert, who has written fascinatingly about it in *Ocean Island and Nauru.*)

Dr. J. Ramsbottom has written that *pulque* is much esteemed for its cooling properties, " though the natives also regard it as nutritious " (for reference, see p. 212). Probably, the natives' belief is well founded, but the nutritional value of *pulque* would seem to derive from its contained accessory substances, of microbial origin, rather than from its energy-giving value. The moral of the Mexican story may be variously drawn. You may like to compare that story with those about baby foods in the next chapter.

CHAPTER XIV

MICROBES AND THE HOME

As this is not a textbook of hygiene, no detailed discussion of microbes in relation to woman can be given. Only a few points can be discussed, and in casual fashion.

This chapter may be none the less instructive; for the chief value in the home of having a knowledge of microbiology is that it makes clear the effectiveness of just plain ordinary cleanliness. This is, firstly, because soap and water, or a damp duster, while they do not get rid of the microbes to the point of making things sterile, do remove the decomposable " dirt " on which microbes flourish; secondly, such microbes as exist in an ordinary home are no menace. There are always plenty of microbes about, but (unless there is sickness in the home) they don't matter, if the home is ordinarily clean and the inmates well fed.

One of my colleagues lodged with a woman who was always cleaning out her little ornamental fish-pond, but he averred that she was not nearly so particular about cleaning up the kitchen. This exemplifies a wrong sense of values. There were " germs " in the fish-pond of course, but they were the ordinary harmless microbes natural to water and soil. Even if the pond had been allowed to become smelly, it could have been no worse than a nuisance. Ponds, sinks, and drains do not become foci of disease unless they have been infected from a bearer of microbial contagion. Such places will always be swarming with " germs "—microbes of many kinds—but the germs are normally harmless. No practical mode of disinfection exists that would keep such places constantly free from microbes. The cleaning of over-crowded fish-ponds for the purpose of keeping water fit for fish is another matter, as are some tropical problems

(*e.g.* those connected with malaria). There is normally no need for disinfection of urban household appurtenances—sinks and drain-gullies and the like—on account of a supposed danger to human health.

Microbes are always present in water open to the air. A smelly drain or pond is one in which a lot of decomposable matter is present. The smell indicates the presence of an excess of microbial food, such as a blockage in a drain. Microbes cannot work without food. If enough material is present to give rise to active microbial action, and thus to cause a bad smell, it is not only the fault of the microbes. Smells, while they may be an indication of microbe activity, never carry microbes, deadly or otherwise; consequently a merely bad smell cannot cause physical disease. If you have a fear that smells cause disease, banish the smell by all means, but banish the fear also.

I am not aware that anyone has tried to find out what kinds of microbes exist in a gully taking water from a kitchen sink. Small amounts of fat go down the drain and linger in the pipes. The conditions for a spontaneous enrichment culture are present. I should therefore expect to find several kinds of bacteria able to decompose fat; these might be interesting from a scientific point of view, but would certainly not be harmful. The fear of microbes is one which the sellers of disinfectants and disinfectant soaps make great play. If you wash the kitchen with a disinfectant to destroy germs brought in from the garden, you should ask yourself why it does not seem desirable to disinfect the garden itself, or at least to scrub potatoes and carrots and onions with disinfectant as soon as the greengrocer delivers them. And, of course, you should not have any pot plants: which is absurd! Disinfectants are valuable in sick-room, sanatorium, and surgical theatre, but it is easy to overrate their value in the home.

Plain soap and water is wonderfully effective for ordinary cleansing operations. It is doubtful whether medicated

soaps (such as " household carbolic ") have any special virtue, but they have a smell that to many people is reassuring and therefore pleasant. Probably the disinfectant value of such soaps is about the same as that of their higher-class relatives, the toilet soaps, for the smell of which no claim is made beyond agreeableness.

This brings me on to a brief consideration of the microbicidal value of perfumes. Most of the essential oils and other substances of which perfumes are compounded have a weak bactericidal action, but the proportion of essential oil in a soap is always, and in a bottled perfume usually, small. The usual handkerchief perfume owes most of its antiseptic value to the alcohol which it contains. Alcohol is an effective germicidal only when strong, hence a bottled perfume consisting largely of alcohol needs to be applied almost neat if it is to be antiseptically useful. In recent years many perfumes have come to be made with terpeneless oils, which dissolve in quite weak spirit, and can therefore be used for cheaper perfumes on account of the lesser duty payable upon the alcohol. In Britain at least, price may be a rough guide to the antiseptic value of bottled spirit perfumes, there being no legal obligation on the maker to declare the percentage of alcohol. Spirit perfumes must, to be effective disinfectants, be of high-strength spirit, and be used with little or no water added. Their use can be recommended only as a luxury, or in an emergency in place of surgical spirit.

Comparatively little study has been undertaken of the microbial aspects of household tasks, and what has been done in this line has mostly been done in the United States. A microbiological examination of the operation of dish-washing by hand was recently made by a student of some American university. Several British newspapers, including the literary but not very scientific " Spectator ", thought this was rather comic. I cannot see that there is anything essentially funny or ignoble in looking into the

basic operations of household work. Apparently nothing very profound emerged from this particular study; it was, I think, concluded *inter alia* that the washing-water could not sterilize dishes, since if the water was at a temperature that could be borne by the hand, it could not be lethal to microbes. If I wished to criticize such an enquiry, my criticisms would take the form that—provided no carrier of actual infection is handling the plates or has been among the users of the dishes to be washed—it cannot matter from the health point of view whether the microbes are alive or dead so long as the dishes are made reasonably clean and dry enough.

The washing by hand of the more public crockery and glass of restaurants and bars offers a bacteriological problem of which the seriousness is not certain. It is one to which attention is wisely being given in America; more thought might be given to it over here. In restaurants, too, every toilet should have washing accommodation. Small restaurants are very deficient in this respect. Though strictly it is a digression, I should like to suggest that in chocolate factories and other places in which hands come into contact with fine food, not intended to be sterilized, close attention should be paid to the bacteriological condition of the door-handles, especially about the toilets.

The sanitation of personal brushes and combs is yet another matter. Do you ever disinfect your tooth-brush? There are probably no more germ-laden articles in the house than the tooth-brushes: moist, warm, and not without traces of microbial pabulum!

The ordinary household microbes are the ordinary microbes of soil and air, and they abound anyhow. A few more or less on a plate or cup can harm nobody. An extension of the same idea will show you that dust, as a carrier of "germs," need not be feared (again in the absence of actual sickness, as you will understand throughout that I am writing of the ordinary household, and not of a sick

room or sanatorium). So you use a damp duster or rubber, not so much to keep down the number of "germs," but to save picking up the same dust twice. Removing dust is a neatening rather than a sanitizing practice. The vacuum-cleaner is not an especially sanitary device from the micro-biological point of view. The refrigerator, while effective, is more of a luxury than a real utility.

The boiling of clothes needs no comment. Ironing is an effective method of achieving practical sterilization of linen and similar material, according to American investigations. Cooking, however, is often not a thorough method of killing food-borne micro-organisms. If your cook, for example is a "carrier" of typhoid fever,[1] you are exposed to a high risk of infection from the typhoid bacteria. The central portions of a cake or loaf do not rise during its cooking to a temperature much higher than that of boiling water—if as high. Flour has few bacteria in it; about 10,000 per gram, or saltspoonful, is about the figure usually quoted, but these are merely common saprophytes derived from the wheat-field. Sugar sometimes bears a very few bacteria able to resist quite high temperatures, but these do no more, at the worst, than provide an occasional problem to the canner of artificially-sweetened fruits by causing wastage after "retorting" (as the cooking in the can is technically called).

There are two little points about tinned foods that are not generally known. One is unimportant: it is that sweetened condensed milk is usually not sterile. Though in the processing the milk is heated, the heat is insufficient to kill all bacteria. Sweetened condensed milk depends for its "keeping" mainly on having a high percentage of sugar, which, by binding water into a syrup, leaves none free for bacterial necessities. Therefore, those bacteria and yeasts that are present are unable to grow. They are usually

[1] A carrier is a person who harbours in or on his body micro-organisms of a definite disease but shows no symptoms of infection.

harmless, in any case. A similar mechanism keeps jams and honey good. It is imperfect bottling and storage rather than microbes which is responsible for the spoilage of honey by fermentation. A faulty lid lets air in, the honey absorbs moisture from the air up to a point at which the natural yeasts and other microbes can get busy, but this does not happen with properly-bottled honey. The second point is that there is no obvious microbiological justification for the often-repeated advice to turn canned goods out of the tin immediately the tin is opened. On the authority of Professor Fred W. Tanner, this precaution is needless.

Surprisingly low temperatures in the interior of a spaghetti pudding during and after cooking were revealed during an American investigation into the possibility of bacteria living in home-cooked food. The temperatures were such as to be favourable, not adverse, to bacterial multiplication. Hence, if milk that is already inclined to sour, because of the development of important numbers of acid-forming bacteria, is used for a pudding, the chances are that it will sour completely during the cooking.

It is very easy to make an unwitting demonstration of the effects of bacterial multiplication, by adding fresh milk to fill up a jug containing a residue of day-old milk. I confess I am sometimes tempted to do this, to save washing-up another jug! The old milk will have developed numerous bacteria, and will act as an inoculum for the mixture. On the other hand, the ordinary washing-up process, while not sterilizing the jug, does not leave enough milk-souring bacteria to act as an inoculum worth mentioning. An inoculum must consist of a visible amount of milk for it to be of practical importance.

If you do not sterilize your milk-jugs, or at least heat them to pasteurizing temperature (about 145° F.), I do not see why you should use pasteurized milk. Pasteurization is a form of partial sterilization whereby the majority of the harmless souring organisms are killed, together with

tubercle bacteria, while tougher micro-organisms escape. The purpose of pasteurization of milk is mainly to enable it to keep saleable for a longer time than it would if the souring bacteria were present in large numbers. Therefore pasteurization is a boon to wholesalers because it enables them to " handle " originally dirty milk; it avoids a lot of fuss in buying (such as insistence on standards); it also simplifies selling.

That pasteurization should be altruistically welcomed by a large section of the medical profession, who thus become allies of the merely commercially-minded wholesalers, may seem strange unless one understands medical mentality. Mr. G. K. Chesterton alleged that New York was up-to-the-minute with the latest inventions, such as the telephone, but was poorly served with the invention before the last, such as ordinary letter deliveries. The medical profession is rather like New York as Mr. Chesterton saw it. Where constructive medicine is concerned, the bulk of the medical profession are a long way behind the more advanced of their colleagues; in particular, on the question of the relation of bacteria to disease, the profession has not advanced beyond the stage of regarding the bacterial agents as the whole story in infection. Hence the insistence on killing bacteria in milk.[1]

It seems to me that to advocate pasteurization disinterestedly is a confession of failure to secure decent milk to start with; while to say that pasteurization does not harm milk is to pronounce on the subject of protective qualities of foods, about which our knowledge is by no means com-

[1] Professor Drummond (ref. on p. 126) has given the following example of medical arrogance: " Thirty years ago, in a wave of enthusiasm for the germ-theory of disease, doctors recommended the careful sterilization of all food intended for infants and young children." He gives even more striking examples from earlier years; in one of them, a court physician explained why an artificial food could not fail to agree with new-born infants; he was at a loss to explain why doctors found that the food did give babies acute indigestion.

plete. That commercial interests use pasteurization does not mean any more than that considerations of profit may retard an advance, or even turn back the clock. Not all that is new is necessarily progress, however loudly it be so proclaimed by interested parties.

BOOK

F. W. Tanner, *Bacteriology and Mycology of Foods*, John Wiley, New York: 1919. This and Professor Tanner's other book (mentioned on p. 43) contains a store of readable information about many aspects of microbiology applied to the home.

CHAPTER XV

WHAT MICROBES DO IN THE SOIL

(A little sweep-up)

I HAVE never found in any book a *simple* statement of what microbes do in the soil; therefore, I present this little summary.

Prospective examinees who see in this chapter an unhoped-for opportunity of cramming, should read the opening part of the Epilogue.

This chapter is written exactly in the style I use for the more intelligent parties of visitors who come to Rothamsted and spend a quarter of an hour or so in the Bacteriology Department during their tour. Imagine that you are one of a party of twenty and that you are being addressed from the other side of a central bench. There are no seats, and you have already been talked at, standing, by other specialists in other departments, and there are more to see and hear yet. By the end of your morning, if you are lucky, not more than seven of the eight lecturers will have used the phrase: "As time is short, I will not go into details."

This is the little Department of Bacteriology, of which Dr. H. G. Thornton is Head. This lab. was built in 1906 as the outcome of a bequest by Mr. James Mason, an Oxfordshire landowner who was interested by the striking contribution that leguminous plants made to soil fertility, and wished for more to be known about the importance of legumes in farming and how they acted in building up fertility. Although possibly you associate bacteria with disease and death, I shall tell you no horrors, because we

have none. Our work is entirely concerned with bacteria in connexion with their usefulness in agriculture and their place in maintaining soil fertility. Micro-organisms capable of causing disease in animals, plants, and man do occur in soil, but we are concerned solely with beneficial aspects of soil bacteria, and we seek to adapt their life-processes to practical uses.

There are several important groups of soil bacteria, of which one is formed by the bacteria associated by leguminous plants, but before I tell you about members of these specialized groups, I had better tell you something about the size of bacteria, and about the numbers in which they occur in soil. Bacteria are the smallest organisms definitely known to be living. Their size is usually measured in a unit called the *mu*, a *mu* being a thousandth of a millimetre, and a millimetre being roughly a twenty-fifth of an inch. A bacterium is a round or rod-shaped organism from one to six or ten *mu* in length, and about one *mu* in breadth. So very roughly—twenty thousand bacteria could lie on an inch, and a cubic inch could contain at least a million millions.

There are two methods of counting bacteria in soil. One is to shake up a weighed small amount of soil with sterile water, and dilute a little of that watery soil suspension with more water until we have a suspension containing, say, one part of soil in a quarter of a million parts of water. We then take a little of that very dilute suspension and add it to a plate of prepared melted nutrient jelly containing the sort of foods the bacteria require. The bacteria will be widely dispersed over the plate. After the jelly has set, each bacterium will grow on the spot where it had settled, and in a few days it will have multiplied itself until a visible spot of growth has developed. Such a mass originating from a single bacterium is called a colony. By counting the colonies on a plate, it is a matter of simple arithmetic to tell how many bacteria will grow from a given weight of soil.

That is the old method, and it suffers from the disadvantage that no nutrient jelly we can prepare is suitable for the growth of all kinds of soil bacteria. That method gives numbers ranging from about 20 to 50 millions of bacteria per gram of soil; a gram is about a saltspoonful. Though those numbers may seem enormous, it has recently been found that they represent only about 1 per cent. of the total numbers of bacteria in soil. By counting stained bacteria directly under the microscope, it has been found that there are from two to four *thousand* millions of bacteria in a gram of soil, though not all of them are alive. This last number is so enormous that you may wonder how there is room for anything else but bacteria in the soil. However, even that large number occupies less than a half of 1 per cent. of the soil volume, so there is plenty of room left for the circulation of air and water between the mineral grains.

An interesting thing about the numbers of bacteria in soil is that they do not remain constant, but vary in unexplained fashion almost from hour to hour. The curve here (Fig. 9) shows variations in bacterial numbers in bare soil sampled every two hours (which is as frequent as it is practicable to do it) over a period of 48 hours. You will note that the numbers here (A) are very much less than they were two hours before (B) or two hours later (C). These fluctuations are real, for they considerably exceed the experimental errors, which are calculable. We are quite unable to account for the fluctuations, but they do not depend on rainfall or temperature or any obvious cause. It follows, however, that a single snap count of bacteria can give no direct information about the fertility of soil, since a different though equally true result might be obtained at another time.

The knowledge that bacteria in soil are a hundred times more numerous than they were previously thought to be, does indicate that there is a large labile stock of bacterial

matter in the soil; instead of a few pounds per acre, it is a few hundred pounds per acre, and since an acre can feed one cow on the average, the weight of the micro-organisms in the soil is about as great as the weight of large animals on a similar area of land.

This mass of bacteria can be divided into groups performing more or less definite functions. Most soil bacteria are saprophytic, by which I mean that they can live on dead organic matter. The saprophytic bacteria seize on any dead matter that comes their way, such as fallen leaves, dead stems, animal bodies, and excretions, and plant material, such as the stubble which the farmer ploughs into the soil. Most of them are not fussy about their food, and turn to anything which comes. When a dead leaf or a bit of straw comes their way, the bacteria utilize what they can of it, and (provided they have moisture enough) they multiply enormously in presence of such food, so that the local population in the neighbourhood of a bit of organic matter rises much above even the enormous numbers I have given you. When, however, the food supply becomes exhausted, most of the bacteria disappear—possibly being attacked by other microbes—until equilibrium is restored. I say equilibrium, because the soil population is not static, but dynamic.

There are specialized groups of microbes thriving on special classes of plant or animal constituents. The cellulose-decomposing bacteria compose one of the more important groups. These bacteria break down the cellular structure of plant remains by attacking the cellulose; they derive energy from the process in much the same way as bacteria with less specialized tastes feed upon sugars. Another group of bacteria has the peculiar property of using disinfectants like carbolic acid (phenol) and cresols as energy material; and this rather upsets the notion that carbolic acid is useful for destroying germs and not for encouraging them. These bacteria were discovered here as a result of a

study of the effects of partial sterilization of nursery greenhouse soils by means of coal-tar products, amongst which was naphthalene. From the point of view of general biology, the bacteria attacking naphthalene are of interest because naphthalene is a purely artificial product, not being known to occur at all in nature. Agriculturally, the phenol-decomposing bacteria are of importance because phenolic substances are constituents of urine, and though such substances are decomposed to some extent chemically in the soil, the phenol-using bacteria play a part in preventing an accumulation of them to a point at which they would become poisonous to plants.

So we have broadly two kinds of decomposition going on in the soil: the type of decomposition performed by the phenol-using bacteria, which is really scavenging, and the more important activities of the saprophytic bacteria.

The saprophytic bacteria divide their activities between the two broad classes of substances: carbohydrates and proteins. By decomposing the former they release carbon dioxide for plant use. In the decay of proteins and other complex nitrogenous substances, carbon is likewise restored to circulation, but nitrogen also is made available as plant food. Many kinds of bacteria and fungi are able to break nitrogenous materials down to simple substances like ammonia, upon which most plants can feed. Such microbes are said to ammonify, or to be ammonifiers. Other, more specialized, bacteria convert any excess of ammonia in the soil into oxygenated compounds; this transformation proceeds in two stages, resulting in the production of nitrate. Nitrate is the final state to which nitrogen is brought by microbial decomposition of proteins. It is generally regarded as the form of nitrogen most easily assimilated by plants.

These ultimate transformations of chemically-complex materials into such simple things as carbon dioxide, water, ammonia, and nitrate, provide examples of the linked

progressions and successions which are almost the rule in the microbial world. At each stage, some type of microbe flourishes up to the limit of its income: it is then forced to give way to some other type more suited to the conditions created by the first, which itself is very often almost entirely devoured by its successor; and so on until the microbes can no further go, and subside into a resting equilibrium to be upset by the next accession of dead matter.

This activity in decomposition is tremendously vital because, without it, life on the earth would cease. What the soil bacteria are doing is to prepare food for succeeding generations of plants. Plants require only chemically-simple compounds; they cannot digest dead plants or dead animals or animal excreta, and the task of the soil microbes is to break down such materials into forms which plants can use. This breaking-down is known as decomposition or decay, and since you may associate these terms with something unpleasant, you may be reassured to learn that decay is only an expression of microbial activity in the cycle of life. The soil is not only a storehouse of plant-food; it is also the plant's kitchen; and the soil bacteria, assisted by fungi and protozoa, are the chefs of the underworld. When you let a compost heap rot down, and then dig-in the product, you have separated the functions of preparing and serving plant food.

So much for breaking down or decomposition. The other main aspect of the work of soil bacteria is synthesis, or building up. This is confined to building up nitrogen compounds from atmospheric gaseous nitrogen. Most microbes and all animals and higher plants can make no use whatever of uncombined nitrogen gas; to them it is simply an inert diluent of air oxygen. The ability to seize upon air nitrogen and build it up, atom by atom, into compounds which plants can use is restricted to a few kinds of microbes, of which I need mention only two. One of these is the bacterium known as *Azotobacter*, which is common in

rivers and ponds as well as in soil. It is free-living, that is, it fixes nitrogen on its own account, not requiring the co-operation of any plant. Its contribution to fertility is relatively small, and is not sufficient to permit the growth of a cereal crop of economic size in this country.

The most important nitrogen-fixing bacteria are those which live in partnership with leguminous plants, such as peas, beans, and clover, in little warty masses, called nodules, on the roots. These nodules (see Plate V) are not disease-forms, though they look as if they were. They are the homes of millions of bacteria, which get into the legume roots if the soil first contains the bacteria. The plant supplies sugar, which it forms in the leaves from atmospheric carbon dioxide under the influence of sunlight, and in return the bacteria supply nitrogen in the form of compounds which the plant can use. As a result, the leguminous plants are always rich in proteins, and some of them are valuable fodder crops.

The lucerne plant is one of the best fodders known, but until recently it could not be grown successfully in the North and West of England; and farmers thought that it failed for some reason connected with the climate. Many experiments carried out by and for Rothamsted showed that the reason for the failure of lucerne to grow in the West of England was that its nodule-forming bacteria were there absent from the soil. As a result, the bacteria were sent out after they had been grown in test-tubes in the laboratory. When the bacteria had been applied to the lucerne seed, it was found that lucerne would grow as well in the West and North of England as anywhere else. Bacterial cultures for lucerne " inoculation " are now made commercially, thousands being sold every year, but Rothamsted tests the cultures periodically in order to ensure that they remain up to a standard. It has been necessary to supply not just any lucerne nodule bacteria, but to choose that race of lucerne bacteria which gives the best

growth of the plant and the most protein, for there are differences in the qualities of races of bacteria.

It has been known since Roman times that leguminous crops were not exhaustive, but restorative, of soil fertility, but it is only within the last fifty years that it has come to be known that the action of nodule bacteria lies at the bottom of the gain in fertility following upon the growing of a leguminous crop. Even though the top is grazed or carted away as hay, the legume roots provide the equivalent of a dose of nitrogenous manure for a following crop, while if clover is established among grasses, no nitrogenous manure is necessary except for a few special purposes—but the fertilizer merchants will not thank us for stressing that point! However, in view of the successful attacks of the chemical industry on biological products such as indigo, it may be a portent that biological methods, and in particular the harnessing of soil bacteria, are now challenging synthetic chemical methods.

I will sum up in a few words what microbes actually do in the soil. It is really very simple to understand, once it is properly put to you!

1. *Breaking Down*

Soil bacteria, assisted by other microbes, attack plant and animal remains and break them down into simple substances which plants can use. This is the process known as decomposition, or decay, and is an essential feature of life. The pre-digestion of plant food is performed by unspecific bacteria, or by specialized bacteria capable of attacking particular substances. When the attack is on substances that might be toxic to plants, the bacteria are acting as scavengers.

2. *Building Up*

This is performed by nitrogen-fixing bacteria acting and living free in the soil (*Azotobacter*), or in partnership with

plants (the legume nodule bacteria). The growing of a leguminous crop is a useful aid to keeping up fertility under farming conditions.

3. *As a Whole*

There is an equilibrium between small forms of life in soil. In resting soil, bacteria greatly outnumber the other forms of life. When decomposable material is present, the equilibrium alters. The soil microbes are essentially a population of workers capable of multiplying themselves rapidly whenever there is work to be done. The microbes themselves are liable to decomposition.

The soil microbes constitute a not inconsiderable reserve of plant food.

CHAPTER XVI

BACTERIA AND THE GARDENER

Alice didn't know how to begin a conversation with people she had just been dancing with. "It would never do to say 'How d'ye do?' now," she said to herself: "we seem to have got beyond that, somehow!"

"Through the Looking-glass."

You, questioning: This is just the opportunity I've been wanting. I didn't know there were such people as soil bacteriologists——

I, interrupting: No, probably not. It's an unusual profession, and, as a matter of fact, there are only about half a dozen of us in the whole country, in three or four places.

You: Is that all? Not much chance of rising in your profession, so to speak, by catching the boss's eye! What I meant was that in gardening books and so on, they always talk about bacteria in the soil, and the need for keeping up the soil's stock of humus, by digging in green manure, and so forth, but it never seems to come to much that's any use to me. Just generalities. I saw in a seedsman's catalogue a chatty paragraph which said that without bacteria the soil would be sterile. Is that so, and what does it mean?

I: I confess I don't know what it means. It sounds like a statement of the obvious to me. . . . Perhaps they don't mean "bacteriologically sterile," but that no *plants* would grow? Well, plants *can* grow without bacteria, but only on condition that their food is presented to them in an easily assimilable form, in the way their food is given to them out of bottles of chemicals in a botanical laboratory. The chief function of bacteria and all other microbes in a garden is to prepare plant-food by breaking down plant and animal residues, as I said in the last chapter.

You: Well, I go in for delphiniums (roses, sweet peas, tomatoes . . .) I've read what you have had to say about bacteria and microbes in the soil, and understood most of it, but though it's interesting to know exactly what bacteria do in the soil, I still don't see how the knowledge is any use to me. Can you tell me what I should do to make use of bacteria in my garden?

I: Probably there isn't much you can do beyond what you are already doing. I presume you are using as much stable manure as you can get, and, if you aren't, that you have an old garden with well-manured soil and that you are cashing-in on the fertility of that.

You: Yes, I understand that I must keep the humus supply up. All the books say that, but I haven't grasped why it is so important, nor do I see what the relation is between humus and bacteria. Is there any?

I: Yes, there is, and perhaps I can help you over what is really a misunderstanding perpetuated by some gardening writers. You already know that soil microbes decompose organic matter, such as leaves and straw; they do so in a succession of stages, some of the microbes being decomposed in turn all along the line. At each imperceptible stage a little of the rotting organic matter is converted into an almost indecomposable black substance or set of substances. That is humus, and is the final organic product of microbial activity in soil. In the compost-heap or manure-pile there is not time for all of the material to be converted into humus. A large part of a compost-heap or pile of made manure consists of organic substances broken down far enough to be of use as food for plants with no or little further change. When you dig in such fermented organic material, you are supplying plant food and organic material primarily. You are supplying some humus, and more will be formed as your organic matter slowly changes in the soil. Humus, then, is more truly a by-product of microbial activity than a microbial food or necessity. What your gardening

preceptor means by advising you to " supply humus " is that you need to supply organic matter for the sake of lightening the soil, retaining moisture, and the other advantages it brings.

You: You mean I don't really need humus in the soil?

I: No, I wouldn't say that, because a black, well-manured, old soil, rich in humus, is undoubtedly the best. But since even the richest of such soils can be " worked out " in time, you need to supply organic matter to keep it in trim, as well as to meet current needs of your plants. May I put it this way—that you can hardly hope to add much ready-made humus to your soil, but what you do need is organic matter, the precursor of humus.

You: Can't I use bacteria more directly? For example, could I grow better peas or sweet peas by using some bacterial culture like that sold for lucerne?

I: That's not very likely. There is no evidence that peas or beans of any kind fail to develop useful nodules in Great Britain. If you can't grow peas in your soil, there is probably some other cause than a deficiency of the right sort of nodule bacteria. Your best plan to encourage leguminous plants is to grow them in the rich, limed soil beloved of the gardening writers, and give them plenty of phosphate and potash. Super., or basic slag, and sulphate of potash are good. If you make the soil conditions right, the nodule bacteria, like any other bacteria, will look after themselves. But because the nodule bacteria don't form nodules to fix nitrogen until three weeks or so after the seed has germinated you may—purely as a luxury matter—assist the early growth of the plant by giving them a small dose of nitrate of soda or nitrate of potash a few days after sowing, so as to give the plants a good start and hasten maturity. Don't overdo it—an ounce per yard run will be enough—and once will do, unless you aim to supersede the nodule bacteria altogether.

You: Thanks. But tell me, isn't there a bacterial inoculation that I can put on a heap of straw or grass cuttings to rot them down?

I: No. There are quite enough microbes on any agricultural or garden waste to do all the rotting necessary if only they are provided with the right conditions. The idea of a bacterial inoculation still lingers, but no such inoculum is in the least necessary. If you supply water and food, the existing microbes will do the rest.

You: But your microbes are being sidetracked for rotting down garden waste. Somebody told me of a stuff you put on garden rubbish, and the makers said their method was purely chemical decomposition.

I: If that claim was made, it was nonsense. Bacteria don't need any but the simplest sort of chemicals to rot down organic matter, and in fact "Adco" and preparations made in imitation of it are substantially mixtures of chemical fertilizers. Together with water, they supply the inorganic needs of the microbes which are then able to effect a microbiological decomposition by attacking the grass cuttings or straw or whatever you use. A chemical decomposition of organic matter is used only in, say, paper-making, when esparto grass is boiled with strong chemicals, but that doesn't give a product having any agricultural value.

You: So you can't give me a single instance of bacteria being useful in the garden?

I: I can't, of bacteria or microbes as such, if you're wanting them out of a bottle, so to speak. But if you are composting your garden waste, or are importing straw for the purpose, you are making the best single use of the microbes. Composting your waste instead of burning it may not save very much, except a coughing fit for your neighbour; you don't lose phosphate or potash by burning your rubbish, and the amount of nitrogen you lose is not financially serious in these days of cheap sulphate of ammonia. But you will be losing organic matter, and that is

a plant requirement which nothing but microbes can prepare in suitable form.

You: Is " Adco " or something of that sort essential—I mean, can't I make my own composting mixture of chemicals? Could you be so kind as to give me a formula, since you know what the bacteria want?

I: You don't necessarily need any chemicals at all. If your waste is exceptionally rich in nitrogen, being composed, say, of young grass cuttings with a lot of clover (though I don't suppose you have that sort of lawn, as you are wide-awake enough to use sulphate of ammonia to discourage clovers) your heap will rot down by itself if you wet the grass while stacking down, and keep it moist. But if you use straw, or other materials very poor in nitrogen, you will require to add nitrogen in order to get the carbon-nitrogen ratio close to ten to one. You can add nitrogen in any form you please, but cyanamide or nitrochalk is as good as any, since it contains lime. You will want about six pounds of nitrochalk or cyanamide per hundredweight of dry straw, or say one and a half pounds of either substance per hundredweight of partly-wilted green waste. You must steep the straw, or the waste, or spray it with water, before you sprinkle the cyanamide over it. Make the heap in layers. After a month, having kept it moist, turn the heap, and keep it well moist. A shallow pit is perhaps better than a heap, as it prevents the mass from drying out. I'm afraid I can't give you a formula to suit all cases, because the ruling factor is the composition of your raw material. If you prefer to use sulphate of ammonia, use the same amount as for cyanamide.

You: Does composting get rid of germs of plant disease?

I: It will, provided that the microbial activity in the compost is intense enough either to kill all the disease microbes by heat or to decompose them. That depends largely upon your thoroughness and skill as a maker of

compost, so, if you are in doubt, burn your diseased plants. Burn diseased prunings in any case; microbes do not readily decompose wood, and woody cuttings are undesirable in the compost heap.

You: Should I disinfect the compost-heap at any stage?

I: You cannot, without using an impracticable amount of disinfectant. A properly-prepared compost heap containing no meat or animal refuse will not smell, or attract flies or other vermin.

If you add any ordinary-sized dose of disinfectant, you will not succeed in sterilizing the heap; you will merely alter the balance of conflict between the groups of microbes. If you use an oxidizing disinfectant, such as permanganate, it will burn away an amount of organic matter proportionate to the dry weight of the disinfectant, and inasmuch as the disinfectant destroys carbonaceous matter, the effect will be similar to that following an addition of nitrogen, though more wasteful. Permanganate will stain fresh straw brown, but do not be deceived: that is merely a stain due to compounds of the metal manganese, and does not represent humus!

If you use a coal-tar disinfectant, the organic matter in the disinfectant will only serve as food to some of the microbes—again unless you add a great deal of disinfectant.

But supposing you do add enough disinfectant to sterilize the heap, you will completely stultify your purpose; which is, to induce *live* microbes to rot down the plant materials!

You: Isn't green manuring good for maintaining the soil's stock of hu—I mean, organic matter? Digging in mustard or vetches, and so forth?

I: Yes, it's quite sound in principle, but I don't think it's thoroughly understood yet, even by agricultural scientists. It certainly supplies organic matter, but since the carbon-nitrogen ratio of a whole plant is seldom near ten to one except in very young plants, the rotting of pure green manure in the soil seldom proceeds under ideal con-

ditions. There is usually an excess of carbon, and, in presence of so much carbonaceous matter, the soil microbes proliferate fast, and lock up the available nitrogen in their bodies. They then stay put to a large extent, and fail to decompose with sufficient rapidity to release nitrogen for the plant. Hence, the digging in of green manure, or old turf, may actually result in a shortage of available nitrogen for the following crop. I suggest that when you dig in green manure, you scatter a little cyanamide over it first—say seven to fourteen pounds per rod. Do the same for turf before digging it in, unless you prefer to manure it generously with well-rotted stable manure first instead. As a corollary, never dig in unrotted straw or any other nitrogen-poor material without a generous supply of artificials, including two or three times as much cyanamide or other nitrogenous fertilizer as the amount I have just mentioned.

You: What about hop manure? Is that any good?

I: Yes, if it's a sound brand. Any organic manure assists in keeping up the soil's condition, and a good brand of hop manure has its amounts of nitrogen, phosphate, and potash artificially adjusted to something like correct values. You may also use shoddy, fish-meal, and many other things: the only question is whether they are worth what they cost you.

You: Just one more question, after all this talk of decomposition. Why don't the soil bacteria decompose seeds and living plants?

I: We don't know why dead plants are so easily decomposed, while living ones are not, except by specialized disease organisms. Most of the soil bacteria are saprophytic, which means that they live on dead organic matter, but that, of course, is merely an asseveration and not an explanation. Seeds, however, are decomposed quite readily if sown at the wrong time or under unfavourable conditions. That is not surprising, if the seed is already

dead, but even a living seed may not come up at all if sown in winter: it will decay before the weather is suitable for it to grow. It does seem rather queer that sulphate of ammonia helps grass cuttings to rot but helps live grass-plants to grow !

You: Oh, yes, I nearly forgot to ask you about clover on lawns. You did say something about sulphate of ammonia killing off the clovers. Does it do that by killing off the nodule bacteria of clover ?

I: No, it doesn't kill the bacteria, but any form of inorganic combined nitrogen restricts their eagerness for forming nodules. We have gone into that pretty thoroughly at Rothamsted, but I won't bother you with details. Briefly, it seems that if, over a long period, you supply the legume host-plant with combined nitrogen, the plant tends to shut the door on the nodule bacteria, so that they can't get in without difficulty. Those that *are* in seem to get lazy, and don't bother to fix nitrogen while there is an abundance of combined nitrogen for the plant to take up. They also tend to become parasitic on the nodules. On top of all this, the combined nitrogen stimulates the grasses to better growth, and they begin to crowd out the clovers, so that the clovers have a bad time of it all round, and go under.

You: I see. Um. I seem to have heard that you don't recommend any artificial nitrogen for grassland, except for " special purposes." Is my lawn a " special purpose " ?

I: Yes. Sulphate of ammonia is ideal for fancy grasses, as I may call your lawn. When I wrote that bit about not adding nitrogenous manures to grassland, it was agricultural grassland I had in mind. You see, the farmer likes and needs clovers in his grass, but you don't want them on lawns. One of my colleagues (a bee-keeper) put a lot of basic slag on his lawn, and thereafter so much white clover came up that he had to forbid his children to go on to the lawn for fear of them getting stung on the ankles !

So, don't encourage the nodule bacteria too much in the

lawn. And let me remind you that before you start using sulphate of ammonia on the lawn have the mower sharpened, for you will need it. That's not bacteriology—it's just a promise of plenty of work.

BOOKS, ETC.

There is no general treatise on microbes in relation to gardening. Possibly the most rational discussion of the subject is that given by A. Burgerstein under the title "Bakterien, als Freunde und Feinde des Gartenbaues" (Bacteria as Friends and Foes in the Garden), which appeared in the *Wiener illustrierte Gartenzeitung* (Vienna), Heft (Part) 5, pp. 152–164 [22994], so long ago as 1902! I have not seen the original of this, which is, of course, much out of date.

CHAPTER XVII

MICROBIAL ASSOCIATIONS AND SUCCESSIONS

"*One boy's a boy, two boys are half a boy, and three boys are no boy at all!*"

Saying about boy labour in agriculture, quoted by A. H. Savory in *Grain and Chaff from an English Manor* (Blackwell; Oxford: 1920).

YOU will have noticed that I have repeatedly mentioned happenings in which sets of microbes appear to follow one another in a chain-like progression. The self-heating of damp hay seemed so illustrative that I brought an outline of it into the beginning of our discussion of the habits of microbes. I did this to give you an integrative view of microbes. The textbooks give almost exclusive attention to the non-natural pure-culture view.

I have also briefly mentioned some natural cycles, of which the most fundamental and embracing is the cycle which we may call the plant-food cycle revolving about the soil surfaces. This cycle appears above the soil as the vital activities of plants and animals, and in the soil as the expression and result of the activities of soil microbes. A natural cycle necessarily goes to completion.

Microbial successions and associations differ from cycles, in not being complete. Indeed, it is doubtful whether there is in nature any true cycle of solely microbial *activity*, though there are cycles such as the life-cycle of the malarial parasite and of the legume nodule bacteria, which involve only the *forms* of a given species of microbe.

The production of a pure culture is always the first aim of the laboratory microbiologist, for only when a microbe is obtained in pure culture can its definitive study be undertaken. Such manifestations as: the behaviour of a microbe (by which I mean, of course, a species of microbe) towards

sugars; its ability to fix nitrogen, or perform other specialized acts, can only be determined with exactness when all other species are absent. This is practically a truism.

No detailed prediction can be made regarding the behaviour of mixtures of microbial species. For one thing, only comparatively few mixed cultures have been studied with the view of finding out how the fact of mixing affects the individual species in the mixture.[1] The cases that have been observed can scarcely be said to permit generalities being made. The results of mixing microbes are frequently very different from those which might be expected on superficial consideration. A simple mixture was studied by Marshall.[2] Only two kinds of bacteria were concerned in it. These two kinds of bacteria occurred in dairying operations. One was a commercial "starter" used for souring milk, which means that it was a producer of acid from milk-sugar. The other was a peptonizing bacterium, that is, one which when grown in milk attacked the milk proteins and produced not acid but alkaline compounds, like ammonia. It might have been suspected that a mixture of equal amounts of these bacteria in milk would not give rise to much free acid or alkali, since the products of one would about neutralize the products of the other. Marshall found, however, that when the two were grown in mixture in milk, the result was the production of about twice as much acid as the effective acid-producer could form alone, under conditions otherwise the same.

I suppose that the ammonia-producing bacterium set nitrogenous compounds free, which the other species

[1] The excellent paper by Reese Vaughn, "Some Effects of Association and Competition on *Acetobacter*" (*Journal of Bacteriology*, 1938, **36**, 357 [11056], includes a list of references from which most other references to the literature of associated growth of microbes can be derived.

[2] C. E. Marshall, *Zbl. Bakt.*, Abt. II, 1904, **11**, 739; for additional references on related points see *ibid.*, 1908, **21**, 7 [23684].

" mopped up " in its growth, and so was enabled to grow well, and produce more acid.

Let me make it clear when I use such expression as " a microbe " or " two bacteria," I mean, not one or two single organisms, but one or two species. Once this is understood, it will be seen to be a convenient way of avoiding the insertion of " species of " or " kinds of " into every such phrase. Conceivably there are interactions between two literally single organisms of the same or different species, but no method has been evolved for studying such minutiæ: it is only when the numbers of organisms are very large that we are able to discuss the effects of one species on another. I shall not discuss here the effect that mere numbers of micro-organisms have upon the " average," or the colonial, behaviour of individuals of a single species in pure culture; that is an affair of population-density alone. In mixed cultures, also, it is usually possible to appreciate only population-effects, though sometimes effects upon individuals are appreciable.

Mixing of microbes sometimes has the effect of bringing about a process that might not otherwise occur: the life-processes of the several kinds may be mutually or one-sidedly helpful. (In saying this, of course, one may be consciously or unconsciously anthropocentric!) Thus, *Azotobacter* requires a carbohydrate before it can fix nitrogen. It cannot attack or make use of cellulose (filter-paper), and, if given cellulose instead of sugar—all other essentials being supplied—it will fix no nitrogen. If a mixed culture of *Azotobacter* and a cellulose-decomposing bacterium are put into a medium containing cellulose and other necessary substances, but no sugar, the cellulose-decomposer may break down the cellulose into sugar which the *Azotobacter* can utilize, after which the latter can fix nitrogen. The nitrogen fixed by *Azotobacter* might then be utilized by the cellulose bacteria, and so the processes might be mutually helpful so long as the cellulose lasted.

It would certainly call for a tactful choice of partner for *Azotobacter*.[1]

In fermented milk drinks which are not simply soured—that is, those in which the flavour depends upon something besides lactic acid—mixed cultures are employed. Such cultures consist of a yeast able to ferment the milk-sugar (lactose) to alcohol, either directly or with assistance from the bacteria, as well as one or more bacteria to produce lactic acid from another part of the milk-sugar. The flavour depends upon the amounts of alcohol, acid, and carbon dioxide: the last being present in sufficient amount in the Russian *kumiss* to cause the drinks to froth. The flavour also depends upon the kind of milk used: cow's, mare's, ewe's, etc., and upon the composition of the milk, this not being constant even in the milk of one species of animal. Secondary flavours are imparted by decomposition-products of the fat and proteins, as well as the lactose, of the milk. In the formation of flavours quasi-accidental contaminating microbes introduced by dirt on the vessels no doubt play an important part under primitive conditions.

Not only milk can be fermented by mixed cultures of the yeast-bacteria type. There are little nodular masses variously known as "Jerusalem bees,"[2] "ginger-beer plant," *graines vivantes*, and other names, which occasionally crop up and are popularly credited with remarkable powers of producing a wonderful wine from sugar solutions. Like "kefir grains," which have a somewhat similar appearance, but are used to ferment milk into kefir, these little masses are wholly composed of microbial tissue, and consist of an association of a yeast with one or more bacteria.

[1] Mr. E. H. Richards has allowed me to see his interesting but unpublished data about the similar association of *Azotobacter* with a starch-decomposing bacterium. He examined the effect of temperature too. His results are being published in *Journ. Agric. Sci.*, 1939, **29**. [10966].

[2] Or, as the Rolfes have said (p. 155 of their book, for which see p. 43 of this): "bees of almost any locality sufficiently remote to render verification difficult."

Even tea can be fermented, after sweetening, by a kindred preparation. Dr. J. Ramsbottom, who has given an interesting account (ref. on p. 212) of many such microbial curiosities, dryly says of such fermented tea that "the attempt to popularize it in Ceylon has not been viewed with favour by the excise authorities." This tea, or its inoculum, may be the same as the alleged Chinese (? Indo-Chinese) *kombu-cha*.

Mexican *tibi* (which has very recently been studied afresh by H. D. Mayer [1]) is still another association of this sort, widely used in Mexico to produce a sparkling, slightly acid drink. I have been unable to find out whether this drink is identical with the *pulque* discussed in Chapter XIII. The special interest of *tibi* is that it occurs naturally in small lumps on the prickly pear (*Opuntia* sp.). *Graines vivantes* were probably *tibi* that penetrated to the notice of the Parisian public about 1890.

Tibi, and the other microbial associations mentioned in the last paragraph but one, are all regular associations, by which is meant that they are curiously constant in composition, each being composed essentially of a certain yeast with one or several definite bacteria, and that the simultaneous presence of all the microbial components is necessary to produce the desired fermentation. The association is not an accident, though adventitious micro-organisms may, of course, be present in relatively small numbers. It is noteworthy that the bacteria are often joined into filamentous (thread-like) forms, the threads being covered with a gelatinous substance which the bacteria appear to produce as if it were a sort of protection against the yeast with which they are associated. The thread-like form of the bacteria may also appear as an insurance against separation from the yeast cells, about which the threads are entwined, thus imparting coherence to the mass. I am tempted to suggest that a filamentous form is an invariable

[1] *Das "Tibi"-Konsortium* : Thesis, Univ. Utrecht, 1938.

adaptation of bacteria that are not normally conjoined, but become so when associated with another order of organism (a yeast in the "ginger-beer plant," etc., and a higher plant in the case of the nodule bacteriâ). *Azotobacter* does not form threads, even when associated with protozoa, but it is hardly a typical bacterium.[1]

In our civilized life the most important microbial association and successions, apart from those existing in dairy products, are those which occur in the purification of sewage, and, also, strange as it may seem, in the purification of drinking water.

To take the latter first. Water intended for urban use is passed through what are called "sand beds" or "sand filters." The latter term is really a misnomer, for even the finest sand is too coarse to effect any but the grossest sort of filtration. "Sand filters" are, however, used as one of the last stages of purification, when the water is already clear and bright, and they are used to remove such microscopic objects as bacteria! The sand beds do this effectively because, after the water has been slowly passing through the sand for a few days, a coherent film of microorganisms (mainly composed of tiny plants such as algæ and diatoms) spontaneously colonizes the sand grains, soon forming a film on and between the uppermost grains. This film is soon dense enough to arrest even bacteria. The water that passes through before the film is formed is, of course, not put into circulation until it has also passed through a properly operating filter-bed. The algæ and diatoms of the films live at the "bottom" of the water, but get their carbon dioxide and other requirements from what is already dissolved in the water passing through. A very similar formation of an algal film occurs in Indian "wet-

[1] Those who are interested in this recondite subject should study Mayer's work, in conjunction with the fastidious investigations of H. Marshall Ward, *Phil. Trans. Roy. Soc. Lond., B.*, 1892, **183**, 125 [16192].

rice" fields. These fields are inundated and the water slowly drains through; an algal film forms on the soil surface, contributing much oxygen to the plant roots, to which atmospheric oxygen has no access. In both the foregoing cases, the algal film is formed under shallow or slowly seeping water. Under deep stagnant water an algal film will not form, either on sand or soil.

Sewage purification is primarily a microbial process. Very diverse micro-organisms take part in the purification of sewage. Sewage is relatively highly nitrogenous, and is capable of supporting many kinds of life. It does support many kinds of life at various stages of its purification; and not only microbes, but also saprophytic worms commonly do, and flies may, appear in sewage "beds" and tanks. The remarkable thing is that these organisms—big and little—appear only at certain stages of purification. So true is this that the occurrence of certain flies is regarded by the men in charge as evidence of something wrong: for, if the purification process is working properly, these "indicator" macrobes will not appear at all. This is not a matter of concentration or dilution of the sewage so much as of conditions: for example, sewage is relatively highly concentrated when it enters the purification works, but is not in a condition to serve as the substrate for organisms which may rightly or wrongly appear at a later stage of purification.

The fact that undesirable flies (for example) appear, as it were spontaneously, to infect the sewage, *but only when certain conditions are presented to them by the sewage*, is another illustration of the point I have already tried to make in Chapter IV—namely, that the resisting powers of the medium play at least as great a part as do the attacking powers of the infecting or infesting agent. This example from sewage makes the point very clear, for sewage might be supposed not to have any natural immunity or defence mechanism, as a living animal has. A nucleus of the flies is present everywhere, but the numbers do not rise so much

as to cause a " disease " (nuisance) unless the condition of the sewage is such as to cease to keep the potential invaders out. A further (macrobial) example will drive the point home. If you consider the vegetation on a piece of grassland, and ask yourself why only a limited number of species and not every imaginable grass, weed, and shrub, are growing there, you will probably conclude that the state of the soil and the consequent occupation of the territory by floral species suited by that state, are much more important in determining the vegetation than are the seed-bearing activities of birds and the wind. An adjoining piece of ground may have a different association (sometimes called a consociation) with some entirely different plants, which will fail to appear on the first piece. Even the sowing of " foreign " seed will not alter the flora until conditions of soil and vegetative cover have been radically changed. Park Grass experimental meadow at Rothamsted offers many striking demonstrations of the dependence of botanical make-up of grassland upon nutrition. To those who have seen this meadow, its main lesson is unforgettable.

Throughout the operation of the sewage purification process, the sewage organisms adjust themselves with considerable closeness to the conditions at every stage. Organisms arise, as it were " spontaneously," whenever the state of the sewage suits them, and with equal rigorousness they die out, or fail to appear, wherever the purification has gone beyond, or has not reached, a stage to suit them. If by some mischance, the purification becomes imperfect, so that an unduly crude liquor reaches a part of the works not designed to deal with it, the proper organisms are replaced by new ones, some of which, like the flies, may be big enough to be easily seen and thus to give warning to those who know !

Reverting to my proper subject, the microbes, I subjoin a brief outline of the " indicator " microbes in sewage. The kind of association is much the same whatever

oxygenating method is used to purify the sewage. In the activated sludge process, the sludge is successively composed of the organisms appropriate to the degree of purification. In other processes, such as those in which the sewage is passed through slate beds and trickling filters, the organisms are to be found either in the liquid or as a film on the slate or coarse filter material. On trickling filters the film of organisms acts in a similar way to that of the algæ on the sand beds used for water purification: it is the organisms, and not the supporting materials, which do the work of filtration or purification.

The strength of sewage is expressed in terms of the "biochemical oxygen demand"; the liquid which has the most solid matter demands the most oxygen to oxidize the complex solid matter to simple innocuous substances such as carbon dioxide, water, and nitrate. As the sewage becomes purer, the "oxygen demand" becomes smaller. As the oxygen demand varies, so do the microbes vary in kind. This is well shown in the accompanying table.

STRENGTH OF SEWAGE IN RELATION TO PREDOMINANT ORGANISMS [1]

(Approximate biochemical oxygen demand in conventional units)	*Organisms*
140	(? Intestinal microbes) rapidly giving place to the air- and water-borne:
120	Fungi.
100	Yeasts.
80	Actinomycetes; sheathed Bacteria: *Cladothrix* (*syn. Sphærotilus*)
60	Bacteria (*e.g.* discrete rods, "spirochætes," vibrios); Protozoa (flagellates and amœbæ).
40	Protozoa (ciliates: *e.g. Paramœcium, Colpoda, Stylonychia, Chilodon, Gastrostyla*); and Eelworms.
20	Bacterial clumps ("flocs") *e.g.* of *Micrococci*; Protozoa (ciliates: *Aspidisca, Epistylis, Vorticella, Carchesium*).

[1] After N. W. Barritt, *Rept. Proc. II. Int. Cong. Microbiol.*, London, 1936, 246.

The outline given in the table must not be received too literally.

At strengths of sewage between those mentioned in the table, the distinctions between the groups of organisms are never, of course, abrupt; they shade gradually into each other. Bacteria of some kind are present throughout.

Sewage purification presents combinations of association and succession—successive associations, if you like—the associations, however, being apparently much looser than those in kefir grains and the "ginger-beer plant." We may take as an example of a short succession the microbiology of the surface of Limburger cheese, as recorded by Kelly in New York State:

"The microbiological changes showed a very definite sequence with advance of the ripening period. Budding yeast cells appeared after two or three days and were very abundant from four to five days; at that stage the surface of the cheese became slimy. Uniform distribution of the organisms in the slime over the surface was accomplished by rubbing the cheeses with the hands. Short slender rods (*Bacterium linens*) appeared about the sixth or seventh day, rapidly increased in number, and were uniformly distributed over the surface about the eighth day. Undoubtedly these organisms were responsible for the reddish colour which appears on the cheese at this time. The slime on the surface also became heavier or thicker at this stage. Yeast cells decreased in size from the tenth to the eighteenth day and finally disappeared entirely. It is believed that the other types of organisms, while present from time to time, do not have any important part in the ripening of Limburger cheese." [1]

Another succession occurs in compost heaps, and probably generally during the rotting of plant material. Very briefly the story is that fungi are predominant at first; the

[1] C. D. Kelly, *Journ. Dairy Sci.*, 1937, **20**, 239 [11413], after *Exp. Sta. Record*, 1937, **77**, 693 [8096].

fungi then break down and are themselves decomposed by bacteria. The fungal stage may be made evident by withholding water, after first wetting the heap to allow the fungi to develop. If the heap is then partially dried out, the bacteria cannot get on with their job of decomposing the fungi, which are left as visible mycelial strands.

What is perhaps the most appealing example of a succession is not evident to the unaided eye, and will have to be taken on trust by the reader. It is again a sewage succession. When crude sewage is discharged into an otherwise clean river, the microbes in the water take part in a natural purification, which progresses for some considerable distance along the stream. This natural purification is attended by those same indicator organisms that have been listed above as occurring in sewage works. It is a strange thought that as one walks downstream from a point whereat pollution is received by the river, the microscopic flora and fauna change with every step one takes.

A—G: SEVEN FOR SIXPENCE

Just as Alice was getting quite exhausted, they stopped, and she found herself sitting on the ground breathless and giddy.

The Queen propped her up against a tree, and said kindly, "You may rest a little now."

"Through the Looking-glass."

A

FAIRY RINGS

" *The Rings of* Mushrooms, *which are often seen upon Downs, Plains, and Commons, are called by the Country People,* Fairies-Dances *; these are not always of one Sort of* Mushroom, *but various, according to the Soil that produces them.*

" *It is observable, that the Grass is always much greener and ranker in the Line where those* Mushrooms *appear, than in other Places of the same Grounds, which I have often wonder'd at, as well as the exact Figure of a Circle they have represented.*"—Richard Bradley: *New Improvements of Planting and Gardening*, 6th Edn., London, 1731.

THESE well-known appearances on grassland are due to the action of fungi. The fungi often send up fleshy fruiting-bodies—" toadstools "—which are by no means microscopic. The production of the characteristic dark-green ring of grass is a result of the growth of microscopic mycelium from which the fruiting-bodies arise, so we may be justified in considering these rings, large though they be, as a microbial phenomenon.

Before the origins of fairy rings were known, various superstitions grew up about them. Many of these are discussed in the Rolfe's book (reference on p. 43). The often almost perfectly circular form of fairy rings excited popular wonder, and they were half-believed to be due to a *danse en ronde* of witches or fairies, or else to be the track of a fairy's horse. Another less pleasing explanation was that they represented the Devil's path while churning butter ! Later, a belief grew up that the rings were caused by thunderbolts—whatever those still semi-mythical meteors may be. A belief, held in frugal Holland, that if cows ate grass from a fairy ring the butter would be of inferior quality, seems to have some basis in fact, for it is now known that fairy rings persist only on poor starved land.

Fairy rings originate from a single fungal spore, which is

fortunate enough to find the right conditions for growth after germination. Growth occurs (except for the production of the above-ground fruiting-bodies) entirely in the soil. A dense mass of fungal mycelium is formed, which mobilizes most of the available nutrients and a good deal of the moisture in its locality. As far as the nearby plants are concerned, this mobilization is an immobilization, until the older part of the mycelium decays at the end of a season. The decay (which, you will remember, is performed by other microbial life) releases the plant nutrients from the dead mycelium, and if sufficient moisture is supplied, plant life can resume on the spot formerly occupied by the fairy-ring fungus. The periphery of the fungus goes on growing, probably intermittently, and it must grow *outwards*, just as a tree sends its new roots outwards into fresh soil without doubling them back. Hence, a spot becomes a small circle, and a small circle grows into a ring, of active fungal growth. Colonies on laboratory media are circular for similar reasons.

The rings are not always complete. A large ring caused by one fungus may have other rings, due to another fungus, scattered about inside it, but it seems that nobody has reported two concentric fairy rings.

If the mycelium is all underground, why is the ring visible in the grass when there is no ring of fruiting-bodies to mark, as it were, the fairies' footprints?

A number of people have sought the answer to this. I shall mention only three sets of researches into the question. Lawes, Gilbert, and Warington ascertained that the toadstools from fairy rings at Rothamsted (unmanured plots!) were rich in nitrogen and that their ash was quite remarkably rich in potash. If the mycelium is of similar composition, it becomes easy to understand why active growth of the underground mycelium reduces the amounts of nutrients that would be available to plants, if it were not locked up in the living mycelium. As soon as the fruiting-body or

mycelium decays, however, the accumulation quickly becomes available to plants, which can thus develop better on the old site of the fungus than elsewhere. As the fungal mycelium moves outwards, it starves the plants above it, while its remains nourish for a season those plants which are immediately behind it. Hence, the short-lived superior greenness of the slowly expanding zone or ring of grass that testifies to the microbial life underground.

This is an illustrative picture, but it is not a true one for all kinds of fairy rings. In a ring due to the fungus *Calvatia cyathiformis* studied by Shantz and Piemeisel,[1] the broad zone, in which the growth of higher vegetation was stimulated, was inside the ring of fruiting-bodies marking the outer edge of fungal growth, and hardly any mycelium could be detected in the soil. In a period of ample moisture supply the fungus *Agaricus tabularis* showed two stimulated zones, one narrow one just beyond the fruiting-bodies, and another, broader, inside, but separated from the ring of fruiting-bodies by a zone in which the ordinary vegetation was practically killed. This dead zone lay just above a considerable development of mycelium. Fig. 10 shows the appearance of half the *Agaricus tabularis* ring in section, the centre being to the right.

Shantz and Piemeisel made their observations near Akron, in eastern Colorado. They pointed out that in eastern Colorado the appearance of a ring depends not only on the species of fungus causing it, but also on whether moisture is abundant or not. Less critical observers than Shantz and Piemeisel have given somewhat conflicting accounts of the relative positions of the green rings of stimulated vegetation. Some of them may be right in believing the greener grass to be outside the toadstools,

[1] H. L. Shantz and R. L. Piemeisel, "Fungus fairy rings in eastern Colorado and their effect on vegetation," *Journ. Agric. Research*, 1917, **11**, 191–245, with 30 plates [10965]. Two figures are reproduced from this *Journal* by kind permission of the Chief of Publications, United States Department of Agriculture, Washington, D.C., U.S.A.

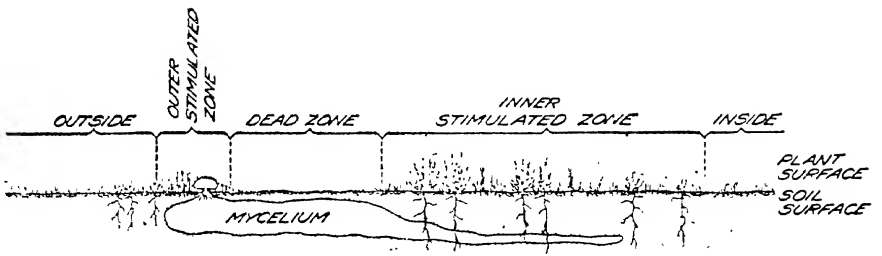


Fig. 10.—A vertical section through part of a fairy ring caused by *Psalliota* (= *Agaricus*) *tabularis*, in grass in eastern Colorado, U.S.A. The centre of the ring is towards the right. The mycelium, which has produced a fruit-body in the outer stimulated zone, is progressing from right to left.
Reproduced, by permission, from the paper by Shantz and Piemeisel.

while others believe it to be inside. Evidently a great deal depends upon the causal fungal species and upon the conditions; and only those writers are wrong who want to fix a single description upon all fairy rings!

Several authors have noted that there is less moisture in the soil of the ring than there is either inside or outside it. Thus Lawes, Gilbert, and Warington[1] found the following results in the now famous Park Grass plots:

PERCENTAGE OF WATER IN FRESH ROTHAMSTED SOIL (STONES AND RUBBISH HAVING BEEN REMOVED) FROM PLOT ON PARK GRASS: CONTINUOUSLY MANURED SINCE 1856 WITH SUPERPHOSPHATE OF LIME ALONE.

Perfect ring, the band of stimulated grass being about 3 feet wide:

Sept. 1877.	*Surface soil.*	*Subsoil.*
Within ring .	22·8	17·0
On ring (centre) .	19·3	13·1
On ring (outer edge) . .	18·5	13·2
Just outside ring	23·3	15·0

Imperfect ring on same plot, band of grass about 2 feet wide:

April, 1878	*Surface soil.*	*Subsoil.*
Within ring .	26·3	19·2
On ring (inside) .	26·3	—
On ring (outside)	22·0	19·1
Outside the ring .	28·0	19·7

Shantz and Piemeisel made similar findings, and discussed the possible causes for the reduction in soil moisture on the ring. They believed that the main cause was that the fungal filaments, not being easily wetted, would not permit the penetration of water into the soil. This "shedding" of water was so marked, that, even in a moist season, the grass cover died for lack of water over a considerable area of an *Agaricus tabularis* ring (see Fig. 10). The honour of first reporting the fact that mycelium of fairy rings was difficult to wet belongs to Jessie S. Bayliss.

[1] J. B. Lawes, J. H. Gilbert, and R. Warington, "Contribution to the chemistry of 'fairy rings'," *Trans. Journ. Chem. Soc.*, 1883, **43**, 208–223 [11107].

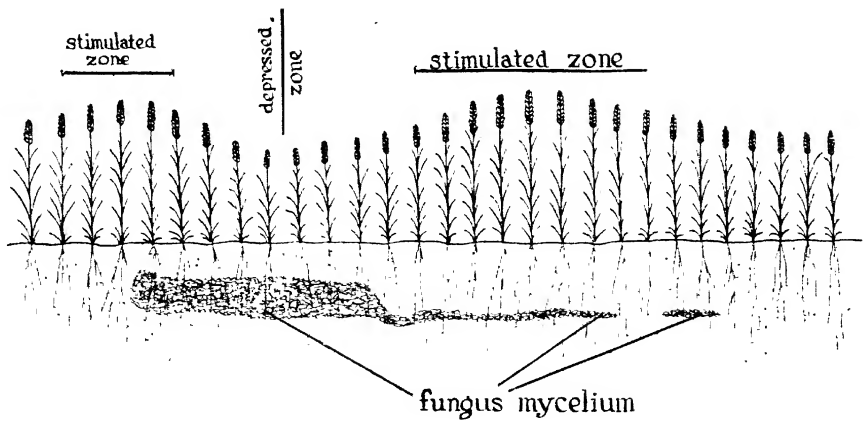


Fig. 11.—Cross-section of part of a fairy ring of the same fungus as that given in Fig. 10, but under wheat, in a year of ample moisture. The wheat plants provide their own height-curve, thus showing the degree of stimulation due to the growth of the fungus inside and just outside the ring. The centre is towards the right.

Redrawn by Dr. H. G. Thornton from a figure in the paper by Shantz and Piemeisel.

In a part of the prairie that had been ploughed up and sown with wheat, the wheat showed by reduced height (and still more by a smaller yield of grain) that it was adversely affected by being above the main mass of mycelial growth of *Agaricus tabularis*. A cross-section of the wheat crop along a radius of the ring would provide its own graph of height, as shown in Fig. 11.

On the other hand, the deeper green of grass in the zones of stimulation gives evidence of there being a larger amount of chlorophyll in a given weight of plant, compared with the plants in unstimulated regions or in the centre of the rings. Shantz and Piemeisel found that in plants in the stimulated zone of the two species of fungi I have named, there was more than twice as much chlorophyll as there was in those outside the ring.

The rate of advance of any part of the Colorado rings was found (during a few consecutive years) to be about 5 inches per annum. Some of the rings had a diameter of 200 feet. In a complete ring of that size, microbial growth would be proceeding over a front 700 feet long and perhaps 6 feet in width, though less than a foot deep. If outward growth had occurred at a uniform rate, the larger rings must have been about 250 years old. So, a microscopic spore can give rise to a structure which has a claim to be considered as one of the largest and not the least permanent of living things.

8

LOOKING-GLASS CHEMISTRY

BOTH microbes and macrobes exhibit an ability to make a selection from a large variety of possible chemical compounds.

This power of choice, or directed chemical force, is expressed in the substances built up by the organism, as well as on the food material it uses.

The most remarkable outcome of this directional analysis and synthesis in organisms is perhaps that concerned with the pairs of compounds of carbon which have their constituent atoms arranged in such a way that one bears to the other the same relation as does an object to its mirror image.

The simplest geometrical solid is the regular tetrahedron or three-sided pyramid. This is enclosed by four triangles having equal sides and angles, and has four points. The mirror-image of a regular tetrahedron is identical with its original.

It happens that each atom of carbon can combine with four atoms of hydrogen, or with four other atoms or groups which are themselves capable of combining with one atom of hydrogen.

Many facts about the intricate modes in which carbon atoms combine with other elements and with each other can be " explained " if it is assumed that the carbon atom is at the centre of a regular tetrahedron, with the four combining-potentialities of the carbon atom each directed towards one point of the tetrahedron.

Now, if all the four atoms with which one carbon atom is combined are alike, one atom being at each corner of the tetrahedron, the perfect symmetry of the tetrahedron is

not disturbed, and the mirror-image of the molecular tetrahedron, however it is regarded, is identical with its original.

If one, or two, of the atoms at the corners are different from the others, the reflection of the resulting molecule is not necessarily the same as its original. It can, however, be made so (in imagination) if the "real" tetrahedron is taken over into Looking-glass Land and given a twist.

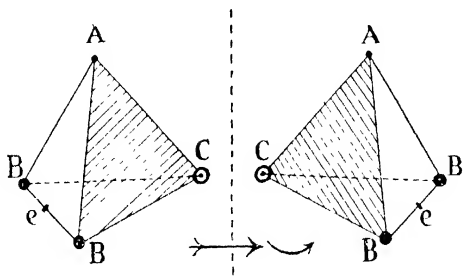


Fig. 12.—Two "carbon" tetrahedra with the same three different kinds (A, B, and C) of atoms or groups attached to the four apices or "corners." Each tetrahedron is the mirror-image of the other, but by suitable twisting, the two can be made to coincide. They are consequently identical.

If each tetrahedron is cut in the plane passing through A, C, and *e* (*e* being the mid-point of the line BB) it will be divided into two exactly equal and similar halves. The plane AC*e* is a plane of symmetry.

The translated original and its mirror-image can be made to coincide exactly, point by point, and line by line, in Looking-glass Land. It is the same thing to say that two four-cornered regular pyramids are exactly superposable in ordinary space, if each has one carbon atom at its centre, and two or three kinds of atoms at the pyramid corners. Such a pair can be made to coincide in every particular.

This is shown (for the case where there are three kinds of atoms attached to carbon) in Fig. 12, which represents

space on both sides of a mirror reflecting the " real " left-hand pyramid. The reflecting surface is indicated by the vertical dotted line. The right-hand pyramid is in Looking-glass Land. The carbon atom is not shown, because only the other four atoms matter in the argument. The four corner atoms are shown as dots of three sizes to represent their three kinds. One face of the real pyramid has been shaded, and it will be seen that the corresponding shaded side is the same in the mirror, but the letters of it run the " wrong way round." (The actual letters must not be taken into considerations of symmetry, of course; they have been attached for reference purposes only.) Hence, the shaded surfaces are not identical, just as the reflected image of a line of print is not the same as the print.

It will, however, be seen that if the left-hand figure is slid over into Looking-glass Land, and turned through one-third of a revolution (atom A remaining uppermost), it will coincide with its image. What was the rear face ACB of the former left-hand pyramid will then be identical with the shaded face ACB in the right-hand pyramid, and the shaded side ABC of the left-hand pyramid will be identical with the rear face ABC of the right-hand pyramid. Identity will be complete. Hence, in a chemical compound, if each molecule is composed of a carbon atom attached to four similar atoms, or to four atoms of two, or three, different kinds, all the molecules of that compound will look and *be* alike. If you still don't see this, please take my word for it.

If, however, the molecules have a carbon atom attached to four atoms all different, the molecules will necessarily be of *two* kinds.

To understand this important and even fundamental duality, look at the " carbon " tetrahedra with four different corner atoms ABCD in Fig. 13. Take the left-hand figure over into Looking-glass Land, and turn it how you will; there is *no* way of making the two figures coincide. These are similar only to the extent that one is the looking-glass

counterpart of the other. They are inescapably two. The tetrahedra are exact mirror images of each other, and are not superposable. If one is regarded as right-handed, the other is left-handed.

Now in a carbon compound having each atom of carbon joined by four chemical linkages to four different atoms, or to four different groups having only one free linkage (and thus behaving like an atom of hydrogen as far as linkages are concerned), chance tells us that, in the long run, half the

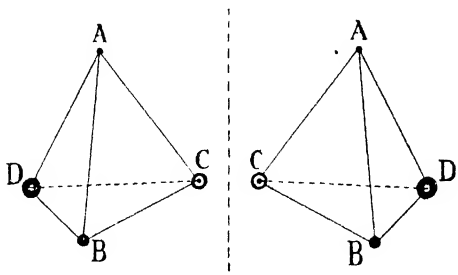


Fig. 13.– Two carbon tetrahedra with the same four different atoms or groups at the apices. Each tetrahedron is the mirror-image of the other, but they cannot be made to coincide: they are different.
Microbes can distinguish between these two kinds of molecules.

molecules must be right-handed, and the other half must be left-handed. This is precisely what happens when such a carbon compound emerges during artificial preparation in the laboratory. The usual result is that a mixture emerges in which half the weight of the substance is made up of right-handed molecules, the other half being composed of left-handed ones. The chemist cannot determine any other result in advance; he has methods for separating one kind from another, but only after both have been made. He cannot make the *d* (*dextro*; right) form without at least a

substantial accompaniment of the *l* (*lævo*; left) form, or vice versa.

In living organisms of all sizes many biologically important substances occur in either the *d* or *l* form without the other being present. The polarity of such compounds seems to have a good deal to do with the inner processes of life. It is possible that in higher plants and animals both forms are made, one of them being destroyed as fast as it is prepared. In any case, the one that is left is often the key to a vital door that cannot be unlocked by the opposite form. If the *l*-form is the nub of a biological process, the *d*-form either will not set it in motion at all, or will do so only imperfectly. Sometimes the analogy is brought forward that a real lock cannot be operated by a key fashioned like its mirror-image, but that analogy is neither straightforward nor easy to make so.

The nicotine in commercial nicotine is derived from the tobacco plant and is almost entirely in the *l*-form, the plant having stored it for some purpose unknown, or possibly having used up all the *d*-form in some vital process. The camphor you buy may be pure *d* or pure *l*, if it is natural. Whether it is *d* or *l* depends on the species of plant it comes from. (It is probably *d*.) If it is synthetic, it is always the *dl* mixture.

Other common examples of compounds existing in the two forms having an object-and-mirror-image relationship are lactic and tartaric acids and their salts. Lactic acid is formed by bacteria from lactose (milk-sugar). In pure culture, some kinds of bacteria produce *d*-lactic acid, while others produce *l*-lactic acid, to the practical exclusion, in each case, of the opposite form.

During the fermentation which converts grape-juice to wine, *d*-tartaric acid is formed almost exclusively; there is very little *l*-tartaric acid. The *d*-tartaric acid is present mainly in the form of the half-neutralized acid, potassium hydrogen tartrate, commonly known as "argol" and

" cream of tartar." In the purification of *d*-tartaric acid from wine-fermentation vats, a small amount of a mixture of equal amounts of *d*- and *l*-tartaric acids is obtained; this may be called *dl*-tartaric acid. The three forms—*d*, *l*, and *dl*—are chemically identical, and are distinguished only by their physical properties, such as melting-point and solubilities; in fact, if it were not for the solubility of the *dl*-acid being different from that of the *d*-acid they would both separate out together during the process used for refining fermentation tartaric acid.

Not only the *molecules* of *d* and *l* substances may bear to each other the relationship of object and mirror-image. The actual crystals of a few substances that exist in *d*- and *l*-forms have shapes which are in the mirror-image relation to each other. Carbon tetrahedra having four similar atoms at the corners are perfectly symmetrical; while those having two or three kinds of atoms at the corners are less and less symmetrical. Each of the carbon tetrahedra in Fig. 13, having four different kinds of atoms, or groups, attached to carbon, is asymmetric; this is, they cannot be cut by *any* plane, or be twisted around any line, so as to show equal and similar halves.

Crystals of such *d* and *l* substances, like the molecules of which they are built up, are wholly lacking in symmetry. Ideal mirror-image crystals are shown in Fig. 14. The small shaded faces are responsible for destroying the symmetry of the external appearance of the crystals, and they occur, you will notice, in mirror-image positions. The ideal crystal of such a *d* substance is thus the ideal crystal of the *l* substance as seen in a mirror, and vice versa.

In 1843, Louis Pasteur discovered that from a water solution of the sodium ammonium salt of *dl*-tartaric acid, two forms of crystals could be deposited, and that the crystals of one form had the appearance of the mirror-image of the other. By hand-picking the crystals, Pasteur was able to show that the *d*-form gave an acid identical

with ordinary tartaric acid; the acid in the *l*-form was something then new. This finding was the beginning of a great new chapter in biological chemistry.

Pasteur went further and found that a mould belonging to a species of *Penicillium* attacked these salts preferentially; when the salts were in solution, the mould largely destroyed the *d*-form but left the *l*-form almost untouched.

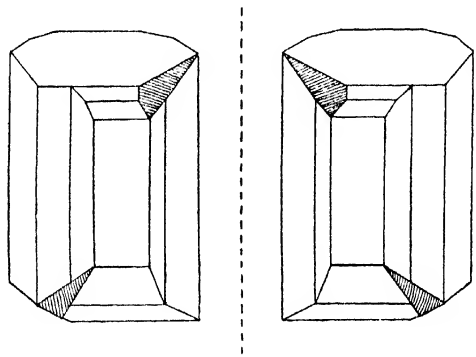


Fig. 14.—"Ideal" crystals of two substances each composed of the same four different atoms or groups combined with carbon into molecules which bear to each other the mirror-image relationship of the kind shown in Fig. 13.

The crystals are also mirror-images of each other; like their component molecules, they are asymmetric. Microbes can distinguish between such substances, even in solution, and can produce or decompose one or the other form to the practical exclusion of the other.

A human being cannot distinguish between the substances (except by very specialized methods) unless they are in the form of fairly large and well-shaped crystals. Pasteur's microbe can reject one and attack the other, even when they are in solution together. This suggests that the microbe, or its enzymes, also possesses a sort of looking-glass discrimination. Numerous examples of this peculiar selective action in microbial processes are now known.

For the sake of simplicity, I have mentioned only a few cases of polarity in chemical substances and in the action of microbes. Sometimes microbes attack a *d*-form, sometimes the *l*-form. It is an odd thought that microbes can have right-handed and left-handed appetites.

NOTE.—In this introduction to the fascinating subject of space-chemistry I have not said anything about what is usually regarded as its outstanding manifestation, namely, the ability of *dextro* and *lævo* compounds to twist the plane of polarization of a beam of light either to the right or to the left. To explain all this would be to pursue a fresh hare, not in my field.

There is a skilful exposition of this aspect of looking-glass chemistry in *The Documents in the Case* by Dorothy L. Sayers and Robert Eustace (Penguin Book, No. 89). The plot of this thriller depends upon the behaviour of right- and left-handed compounds, and, appropriately to my theme, mushrooms are prominent in the story. The scientific details are trustworthy, and are lucidly explained step by step.

A colleague of mine was consulted in a real-life counterpart to the story of *The Documents in the Case*, though in his case it was not murder, but an attempted commercial swindle, that was in question. It was claimed that a secret process had been evolved for artificial production on the large scale of an important but rather expensive substance hitherto obtained commercially only from natural sources, in which it exists as the *lævo* form. A generous sample of the "new" material was offered, and priced somewhat lower than the commercial material. My colleague knew—as you now do—that if the substance really was artificial it must be a mixture of equal parts of the *d* and *l* forms. He suggested, therefore, the same test as was applied in *The Documents in the Case*. We do not know what was done, but, as nothing has been heard of any revolutionary process since, we presume that the sample was of the natural product, bought in the open market, and that the promoters of the scheme were willing to lose a few cents in the hope of making some thousands of dollars.

C

A CRUISE ON THE NORFOLK BROADS

FROM the 7th to the 14th of April, 1934, I went cruising on the Broads in the company of my wife, starting and finishing at St. Olaves, and visiting the greater part of the Rivers Yare, Waveney, Bure, Thurne, and Ant, east and north of Reedham. (See map, Fig. 15.) Although the cruise was intended to be solely a holiday, I provided myself with some sampling equipment, comprising four ointment pots for mud samples, and ten three-ounce narrow-mouthed flat medicine bottles of clear glass for water. Fourteen samples were taken from spots fairly evenly distributed over the area visited. These samples, annotated at the time of taking, were examined in the bacteriological department of Rothamsted Experimental Station shortly, but not immediately, after my return there on the 14th of April, 1934.

The mud samples showed little of special interest, though it may be remarked that a composite sample collected from the shores of two backwaters about a third of a mile below Wroxham road bridge appeared to be unusually rich in diatoms.

The ten water samples were collected singly, without any duplicates, at various spots in the rivers and land ditches. Details are given in the table, p. 193. The samples were kept uncorked (to discourage anærobiosis) and were shaken daily while in the boat.

Six of the samples were not especially interesting. On account of the need for portability, the samples taken were small—about one ounce of water per bottle. Hence, the examination that could be made was restricted. It was confined to a general qualitative examination, a rough

enumeration of viable bacteria, and to a search for the nitrogen-fixing organism, *Azotobacter agilis*. This is a purely aquatic species of bacterium. It was believed to be confined to Holland, and, even there, it has been found only in canals and drainage-ditches. It therefore differs from most known kinds of soil microbes, which appear to be distributed over very large areas, if they are not of world-wide distribution.

The Search for Azotobacter agilis

Shortly before this Broads trip was made, a paper was published by A. J. Kluyver and W. V. van Reenen [1], both of Delft, Holland. In this paper a critical survey was made of the characteristics and reported occurrences of *Azotobacter agilis*, and it was concluded that the true organism had not been found outside Holland. It may be explained that the genus *Azotobacter* includes several species of bacteria able to capture or "fix" atmospheric nitrogen, which, to most living things, is simply an inert diluent of air-oxygen. The genus *Azotobacter* is widespread, *A. chroöcoccum* being much the commonest species. *A. chroöcoccum* is usually assumed to be a soil organism.

In view of the contentions of Kluyver and van Reenen, and also in view of the presumed similarity between some aspects of the Broads and some Dutch conditions, I decided to make a search for *Azotobacter agilis* the prime object of my sampling upon the Broads. This organism is of some interest from the purely bacteriological standpoint, as well as for an understanding of the nitrogen economy of water-courses. *A. agilis* is, as already said, a purely aquatic species. It is otherwise chiefly distinguished from *A. chroöcoccum* by being motile (having the power of proper movement), whereas *A. chroöcoccum* can swim only passively.

[1] *Archiv. f. Mikrobiol.*, 1933, **4**, 280 [1779[6]].

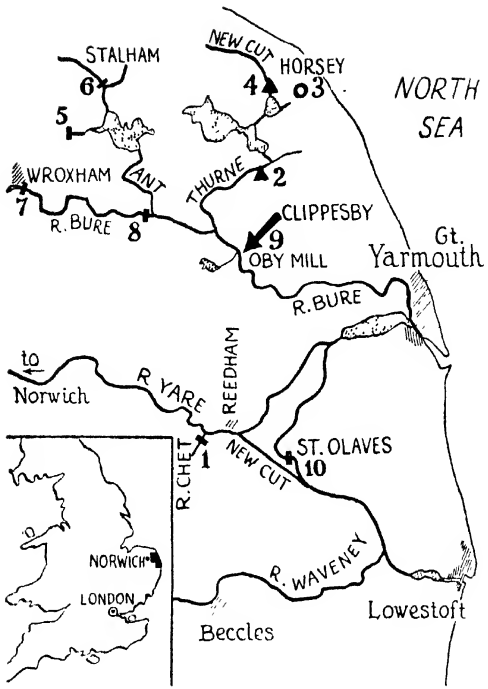


Fig. 15.—Sketch-map of the greater part of the Norfolk Broads district in East Anglia. The inset shows in solid black the area covered by the larger map, with Norwich just outside to the west.

The three triangles (including the large arrow-head) show the sites at which the "miraculous bacillus" was found. The circle shows the site of the land-drain on Major Anthony Buxton's land, a little south of Horsey village. The large arrow shows the general direction of the drainage from Clippesby village, about three miles from the River Bure. This drainage is lifted into the Bure by the old windmill and newer steam pump together known as Oby (or Clippesby) Mill (not to be confused with Upper Oby Mill).

It was in the main drainage channel leading to Oby pumping-plant that the sample was taken that revealed *Azotobacter agilis*, hitherto unknown outside Holland.

For the search, the recommendations of Kluyver and van Reenen were adopted inasmuch as the sampling bottles were prepared with dry mannitol (mannite) as source of energy, and with dry di-potassium phosphate as mineral source. These substances were given in amounts calculated to yield roughly a 2 per cent. and a 0·1 per cent. solution, respectively, in the intended ounce sample of water. The bottles and dry contents were sterilized before departure.

In order that no failure should ensue while at a distance from supplies, preliminary tests were made during March 1934 with water from ponds on Harpenden Common. No *A. agilis* was found, but varying frequencies of *A. chroöcoccum* were encountered, showing that the technique was satisfactory, and that *A. chroöcoccum* was an aquatic as well as a soil species.

The Broads samples almost all showed the presence of *A. chroöcoccum*, and one taken from the main drainage channels of Oby Mill yielded both *A. chroöcoccum* and *A. agilis*. This Oby Mill sample was taken only on the return journey, and the decision was then very nearly made not to bother about taking it !

Upon laboratory examination of the isolated *A. agilis* from the Oby Mill sample of water, it was established that it conformed in all tests made with the description published by Kluyver and van Reenen. Comparison was also made with three strains of *A. agilis* obtained by Kluyver and van Reenen from Dutch canal- and ditch-water, and these strains the Norfolk organism resembled.

The Norfolk strain of *A. agilis* was highly motile, occurred in liquid media mostly as single, refractile cells, and grew well on a nitrogen-free medium made with agar, and containing glucose and potassium phosphate. It grew only poorly on a similar medium containing mannite, instead of glucose, as a source of energy. It possessed well-marked flagella, by the aid of which it was able to swim.

Professor Kluyver has acknowledged the identity of the Norfolk *A. agilis* with one of the Netherlands forms of the species.

Numbers of Bacteria in the Waters

By the plating method, rough " counts " were made of the numbers of micro-organisms in the waters. These estimations were useful for rough comparative purposes only, because of the fact that the addition of mannite and of phosphate had markedly affected the original balance of life. The samples were not strictly comparable, having been taken at different times. Recognizing these facts, I did not proceed with the counts immediately upon return to the Rothamsted Laboratory; this delay had some effect in evening-up the biotic conditions.

After eleven days' incubation at 22° C. on Thornton's counting medium,[1] the colonies that had appeared on the plates were counted on 4th May, and approximate numbers of bacteria per millilitre of water were deduced (see table on opposite page).

The accuracy of the results given in the table does not attain the usual Rothamsted standard, but the choice of the right dilutions to obtain a countable number of colonies had to be a matter of guesswork in the absence of valid ideas of the density of the bacterial population; furthermore, the small size of the samples prevented extensive repetition.

With the exception of those for sample No. 3, the figures for the bacterial counts require little comment. Sample No. 10 was more subject to tidal influence than any other sample. Samples Nos. 3 and 5 were low, and No. 10 was high, in bacteria, but the others showed a bacterial population, under the conditions mentioned, of the same order of magnitude. The peculiar behaviour of No. 3 may be further discussed.

[1] *Annals Appl. Biol.*, 1922, **9**, 241 [1025].

Sample No.	*Site and, in brackets, day of sampling (April, 1934).*	*Bacteria per ml., millions*
1	Midstream, R. Chet, about three furlongs above confluence with R. Yare (8)	178; 108
2	Land drain, east side of R. Thurne, about twelve yards therefrom; about one mile above Potter Heigham railway bridge (8) . .	110
3	Land drain about 100 yards east of Horsey (Private Dyke) Stores; drainage from road avoided (9) . .	0 7; 1 2; 0 8
4	Midstream, Horsey New Cut, about three furlongs above outfall into Horsey Mere, stream very fast, mills working (9) . . .	161
5	Limekiln Dyke, Neatishead, a yard or two below waterfall of Barton Rond (10)	37; 52
6	Midstream, R Ant, about 150 yards above confluence with Stalham Dyke; slow current (11) . .	130
7	Midstream, R. Bure, at southern end of bungalow village, Wroxham (12)	185; 171; 74
8	Drain and dyke near confluence of R. Bure and R. Ant (12) . .	contaminated with mud; not examined
9	In and near main drainage channel, 200-400 yards behind Oby (Clippesby) Windmill (13) . . .	147; 151
10	"Dock" about 100 yards below St. Olaves road bridge, west side of R. Waveney (9 a.m.; 14) . .	400

A Minor Natural Mystery

Sample No. 3 contained a relatively very low number of bacteria even after it had been kept in the comfortably heated boat and afterwards at laboratory temperatures for a long time in presence of mannite and phosphate. When collected, all the samples of water, except No. 8, contained no visible amount of mud, but, within a month or two all, except No. 3, had developed a thick scum of bacteria,

containing many *Azotobacter chroöcoccum*, and algæ and protozoa had also proliferated.

Samples Nos. 3 and 9 were kept in their original bottles, corked part of the time, for fifty-one weeks after collection. By the beginning of April 1935 sample No. 9 had developed much green algal growth and had a considerable amount of brownish deposit. Sample No. 3 was then still colourless and clear, except for a small amount of whitish fluid at the bottom.

The Horsey ditch-water (which did not come from the navigable Private Dyke, but from a land drain on Major Anthony Buxton's estate) thus evinced a long-continued freedom from pronounced bacterial growth in spite of its containing a source of energy for micro-organisms, and being otherwise, so far as could be judged, in favourable condition to support the growth of bacteria. Its bacterial purity, nevertheless, compared almost favourably with many samples of distilled water, in which salts and sources of energy exist only in minimal traces.

The probable explanation of this is that there was a total absence of nitrogen-fixing organisms, and none entered from the air, so that the original and any subsequent micro-flora could not develop for lack of nitrogen. Even the perfectly potable tap-water of Harpenden will usually develop algæ if left in a bottle in daylight for a long time. These algæ do not depend on nitrogen-fixing bacteria, but develop on compounds of nitrogen already present in the water. It is somewhat remarkable, then, that water from Major Buxton's ditch should behave as if it were practically pure. Flooding of the ditch by sea-water has since made it impractical to renew the investigation.

The Occurrence and Distribution of the "Miraculous Bacillus" (Bacterium prodigiosum)

In the present investigation no special search was made for this historical organism (see p. 197), but it appeared on

the plates used for "counting" the bacteria in samples No. 2 (one colony), No. 9 (three colonies), and No. 4 (many colonies). It was thus apparently confined to the north-eastern area of the Broads. The red colonies from sample No. 4, taken in the Horsey New Cut, appeared not only to be numerous, but to have a particularly intense colour.

G. Gorbach [1] has pointed out that the intensity of the colours assumed by *B. prodigiosum* varies with the type and composition of the medium upon which the organism is grown. His known published work refers to the behaviour of only one strain; the later contribution he promised has not been traced by me.

While, therefore, a single strain may give different shades and intensities of red colour depending upon the medium used for its cultivation, there seemed from my Norfolk Broads observations to be a marked variation from strain to strain.

Summary and Comment

I am not aware of any report of the finding of a water in which *Bacterium prodigiosum* comprised a large proportion of the total number of bacteria, as it did in the Horsey New Cut sample.

The rather striking findings of this cursory examination of a small number of samples suggest that the Norfolk Broads provide a rich field for microbiological investigation.

Azotobacter agilis, an aquatic organism supposed to be peculiarly Dutch, has had its "insularity" destroyed. Another species of *Azotobacter*, namely *A. chroöcoccum*—which is commonly written about as if it were purely a soil organism—has been shown to be common in slightly contaminated water. *A. chroöcoccum*, at least, is probably widespread in ponds, canals, and similar bodies of water.

[1] *Centrbl. f. Bakt., Abt. II*, 1929, **79**, 27 [23684].

(You can test this guess for yourselves by using the technique given on pp. 96–103.) If you do, you (who are, I suppose, a beginner in microbiology) will be doing something that hardly any professional bacteriologist has thought of doing. Study of such useful water bacteria as *Azotobacter* has been almost neglected. It has fallen between two stools. The water bacteriologists have been interested only in those bacteria, such as the organisms of normal and typhoid-ridden sewage, which have a bearing on public health. The agricultural bacteriologists have not been uninterested in *A. chroöcoccum* in soil, but have shown very little curiosity about the bacteriology of waters in contact with their soils.

Even the hitherto scarce *A. agilis* may be found to be widespread if only it is looked for. It would be interesting to look for it in the klongs of Bangkok, and in the possibly less picturesque water-courses of such towns as Bruges, Montargis (France), and Venice—where a mildly salt-tolerant form of *Azotobacter* may exist.

As a sidelight, it may be mentioned that the results given in this section have never been published before, because no scientific periodical could be induced to publish the results while they were recent. The non-pathogenic bacteriology of water was unfashionable in 1934; the pioneer encounters difficulties in the scientific, as in other, worlds. Now that the Freshwater Biological Association has established a laboratory at Wray Castle on Lake Windermere, and has appointed a bacteriologist, the study of the non-pathogenic bacteria in waters may achieve recognition.

D

THE MIRACULOUS BACILLUS

" BACTERIUM " might be a better name for this organism, since it does not form spores. It has been known under many names, but the most interesting feature about its nomenclature is the species name *prodigiosus* (*Bacillus prodigiosus*, *Bacterium prodigiosum*). The English word " prodigious " has become linked up with an idea of great size, but the original meaning of the Latin word *prodigiosus* was nearer the English " prodigy "; it meant " miraculous."

The species name *prodigiosus* was given to the organism because of its occasional appearance on the host. It normally grows in bright red, glistening, raised colonies, often having a remarkable resemblance to drops of fresh blood, and there is not much doubt that the resemblance has been exploited at times by priests in Mediterranean countries.

It has long been known that foodstuffs are liable to become spotted red. Of the earliest examples on record it cannot, of course, be said that *Bact. prodigiosum* was certainly the cause, but it seems probable that it or some bacterium resembling it was responsible for such references as that in the dialogue of Lucian (A.D. second century) to the changing of cooked white beans into blood. When Alexander the Great was besieging Tyre in 332 B.C., the army bread was found to have become blood-red inside. The portent greatly alarmed his soldiers, but Alexander's wise men were equal to the occasion, and pointed out that because the " blood " was *inside* the bread, it meant that disaster would fall upon those in the city, and not upon the besiegers !

The phenomenon of the bleeding host was regarded as a miracle; sometimes it was " explained " as due to stabs

Fig. 16.—Stabbing the host to make it bleed (from a woodcut dated 1492).

inflicted on the host by Jews. Many Jews and other suspects have been done to death as a result of this belief. Scheurlen, in 1896, remarked of this harmless saprophyte, that more people had been brought to death by it than by some of the disease-producing bacteria.

Some interesting details on the point have been given by M. Louis Golding in his Penguin Special, *The Jewish Problem*, from which Fig. 16 has been borrowed.

Bact. prodigiosum is quite common, and will grow easily upon damp starchy materials if they contain a little protein and are not particularly acid. For its experimental growth a slice of boiled potato is often used as substrate (the early growth on this and on other solid media is usually white, as the edges of colonies often are). Occasionally, *B. prodigiosum* has occurred on foodstuffs in such a widespread and simultaneous series of outcrops as to resemble an epidemic outbreak. The causes underlying such outbreaks are not known, but no doubt they have their rise in a set of circumstances especially favourable to the spread and growth of the organism: the prevalence of the organism in or on a foodstuff of suitable composition, coupled with storage in a place warm enough and damp enough to enable the organism to multiply. If the bacterium once becomes established in, say, a wooden vessel, it will copiously infect fresh foodstuffs placed therein; it can be realized how the red colour will continue to appear for a long time, and that it may cause much alarm amongst people living under somewhat primitive conditions. I repeat that those who eat foodstuffs reddened by *B. prodigiosum* are not likely to come to physical harm as a direct result; but the reddening is bound to be disturbing, and owing to superstitious fears associated with it, may lead to panic. At the same time I feel bound to point out that people who live under such conditions as give rise to "epidemic" appearances of *B. prodigiosum* must be constantly ingesting other harmless saprophytes to a similar

extent—only, these other organisms do not usually produce a colour; and so go unnoticed! I may, therefore, humorously say that disorder associated with *B. prodigiosum* is eye trouble and not digestive upset!

In 1844 an epidemic reddening of army bread caused agitation amongst French soldiers, while in 1848 red spots were found on cooked potatoes in a house in Berlin. The latter was investigated by C. B. Ehrenberg, because a person had died of cholera in that house, and it was thought that there might be a connexion between the alarming red spots and the fatal disease. It is now known that the bacterium which causes cholera is very different from the "miraculous bacillus." It was Ehrenberg who gave to the red organism a Latin species name meaning "miraculous," and in some form or another his species name has lasted to the present day.

R. S. Breed and Margaret E. Breed suggested in 1924[1] that Ehrenberg's and later names should be entirely dropped in favour of the oldest name of all that has been given to this interesting and much-studied organism: *Serratia marcescens*. It may be remarked incidentally that two other species of red *Serratia*, rather less intensely coloured than *S. marcescens*, have been found on crude sea-salt (*S. salinaria*) and on salted hides (*S. cutirubra*), showing themselves able to grow in presence of the highest possible concentrations of salt, which is usually regarded as a preservative against microbes!

The suggestion that the name *Serratia marcescens* should be revived and preferred to all other proposed names was made in accordance with the tenets governing botanical nomenclature, in which priority plays a great part. The first scientific investigation of the miraculous bacillus was made in 1819 by Bartolomeo Bizio, and it was he who gave it the name *Serratia marcescens*. Bizio was inclined to class the organism as a fungus, but this is in no way to his

[1] *Journ. Bact.*, 1924, **9**, 545. This, and the associated translation by Merlino (below), have been used as the basis of this section.

discredit, for he was working long before the time when bacteria were recognized as subjects of study distinct from fungi. Even to-day many fungologists (mycologists) claim the bacteria as being the lowest class of fungi. Bizio's classification of the miraculous bacterium as a fungus need not detain us, and I would rather give him high praise as a patient investigator able to devise his own methods, for in his day there was no " technique " of bacteriology.

In his letter[1] to " the Most Eminent Priest, Angelo Bellani," Bizio gives some details of the Italian outbreak which began in July 1819, and which led him to search for a living organism as a cause of the reddening of polenta (a sort of porridge made with maize meal). Excitement reached such a high pitch as to demand the intervention of the police department. There was " stupor, and even fear," among the peasants, who ascribed a diabolical origin to what they called " the bloody polenta." Their fear was so great that they would not live under the same roof as that where supposedly supernatural influences were at work, and they turned to the priests, and implored them to banish the evil spirits. " And since ignorance not infrequently is the cause of calumny," the families in whose homes the polenta became reddened were accused of all sorts of sins. All this from a species of bacteria !

Bizio was convinced that the colouring of the polenta was " but a natural effect," and he investigated the outbreak at Legnaro (near Padua), where the commotion was greatest. His first results were communicated privately to " some educated persons," but he also published anonymously in the newspapers; an enterprising publisher reprinted Bizio's letters in a pamphlet, which was sold in the streets. Whether *that* pamphlet also cost sixpence, I have been unable to ascertain.

Bizio's first signed contribution was the 1823 letter to Bellani, quoted above, and it was in this that he named

[1] Translated by C. P. Merlino, *Journ. Bact.*, 1924, 9, 527 [11056].

organism *Serratia marcescens.* He made the curious observation that the richest growth and deepest colour of the organism on polenta was attained—at least in 1819, it seems—when his experiments were performed in places near rice-fields. Indeed, the colouring appeared sooner on the days when water was withdrawn from the rice-fields, and this fact led him to conclude that exhalations from the rice-fields aided the growth of his organism. (They may have been ammoniacal.)

In investigating the phenomena of the reddened polenta, Bizio was not alone, for there was a whole learned commission at work, but the official labours do not appear to be worthy of comment at this day. The amateurs scored heavily. (Bizio was a young pharmacist.) Similar conclusions to Bizio's were reached independently of Bizio, and of the commission, by Dr. Sette, a physician. Sette presented his results to a meeting at Treviso in 1820, but he had bad luck in the matter of publication, and had to wait four years before getting into print. Sette described the organism under the name *Zaogalactina imetrofa*, which is so odd that few later bacteriologists have spelt it correctly. In justice, it should be pointed out that Bizio's, Sette's, and other contemporary publications on this subject are difficultly accessible, and are regarded as out-of-the-way even in Italy.

Bizio placed *Serratia marcescens* in a new genus, which he distinguished by the name *Serratia* in order to recall to "Italians the name of a celebrated physicist, whose memory is neglected, so that we attribute to the foreigner that which exclusively belongs to us. Serafino Serrati was the first who plied a steam-boat on the Arno, and so, whatever be the merits of the claims of those beyond the sea, that of the invention certainly cannot be accorded to them."

Now, when you took up this book on microbiology, did you expect to find in it a contribution to the early history of steam transport?

E

THE PRESENT STATE OF THE GREAT INTERNATIONAL FUNGUS-GUNNERY COMPETITION

THE favourite weapons in this international rivalry are afforded by a fungus which appears to have no common name; its scientific name is *Sphærobolus*. There are other fungi which shoot, but they are greatly outclassed by *Sphærobolus*, of which one of its supporters has said that it is " not only the largest and the most powerful but also the loudest of all fungus guns." [1]

To be fair, I should state that in *Sphærobolus* neither the cannon nor the ball is microscopic. The ball is a very perfect little sphere about one to one-and-a-quarter millimetres (about a twentieth of an inch) in diameter, and this is propelled from a mortar about a fifth of an inch in diameter and about as high. The projectile and gun together form the fruiting-body of a fungus having microscopic mycelium and spores.

Since I have brought fairy rings rightly or wrongly into this book because they can originate from a microscopic spore, *Sphærobolus* has at least an equal claim to recognition here. This will be the more readily conceded since the projectile of *Sphærobolus* is the decider in the gunnery competition, and the projectile is little else but a spherical packet containing thousands of spores. The gun is, as you will have guessed, the mechanism whereby *Sphærobolus* distributes its spores. Some large plants, too, have mechanisms which enable them to project their seeds over considerable distances, but little *Sphærobolus* is able to give

[1] The information about *Sphærobolus* used here has been wholly based on that in A. H. Reginald Buller's *Researches on Fungi*, Vol. V, pp. 279–370 (Longmans, Green; London: 1933).

a good account of itself: as we shall see, the range it confers on its projectile is comparable with that of even the largest plants that violently expel their seeds.

The *Sphærobolus* " gun " employs no powder or explosive, but is a sort of catapult. An animal dropping or a piece of wood is penetrated by the mycelial threads of the fungus: a number of fruiting-bodies, each consisting of an immature gun loaded with one projectile, form on the surface. The covering over the gun draws back so as to leave a star-shaped opening clear above the projectile, and sooner or later the " gun " " goes off."

Before discharge, the projectile can be seen as a little ball lying on a nearly hemispherical or cup-shaped bed. This bed is not a close fit, and the projectile lies loosely on it, in some liquid. If the gun is cut down the middle before it discharges, it will be seen that the cup-shaped catapult bed of the projectile is nothing more than a web of tissue; the rest of the gun is merely a support for the catapult tissue, which is fixed only at the top, at the mouth of the gun.

When the gun goes off, all that happens is that the catapulting cup-shaped bed suddenly turns itself inside-out, throwing the projectile out with considerable velocity. It also ejects the liquid, and makes a perceptible noise ! This turning inside-out occurs with such suddenness that the action resembles that of a powerful spring touched off. Dr. Reginald Buller has estimated that the act of eversion of the cup occupies about a thousandth of a second. The only moving part is the web, and after discharge of the gun the web remains on top of the gun, but inside-out, and looking like a little dome. Each gun can shoot only once.

Dr. Buller has described the *Sphærobolus* gun in great detail, and I have taken from his description a brief account of its way of working in order to make the scoring intelligible.

Sphærobolus has been known for quite a long time, but the fungus-gunnery competition did not start until 1920.

In that year Dr. Buller began to measure the possible range. Until then the ranges had been grossly underestimated: Lloyd in U.S.A. had said that the horizontal range was from 1 to 5 inches (1909); Massee, in England, put it at a foot or more (1911); though a couple of Germans had said much earlier that the range was over a yard. Another German had found the vertical distance attained by the projectile to be about a yard. Dr. Buller's first experiments were made with both vertical and horizontal fire at Kenora, Canada, in September 1920, and he attained a horizontal range of about 15 feet in his first attempts.

International rivalry may be said to have started when Miss Leva B. Walker (U.S.A.) and Dr. Buller (Canada) were both working in the University of Nebraska. The first records sought by Miss Walker were for vertical fire. Dr. Buller, for instance, had noted that when a culture of *Sphærobolus* was placed on a table, several projectiles hit the ceiling 6 or 7 feet above. Miss Walker improved on this method of observation by constructing a celluloid cylinder some 17 feet high, which she installed on a college stairway after putting a ripe lot of *Sphærobolus* at the bottom of the cylinder. Wherever a projectile hit the side of the cylinder, it stuck, and Miss Walker was able to show that two projectiles had reached the astonishing height of over 14 feet. Some of the projectiles which hit the cylinder walls low down would probably have gone higher if they had been discharged from guns set vertically, and not at an angle, as they happened to be.

Miss Walker (U.S.A.) easily holds the altitude record for fungus gunnery—what would be called an " all-time high " in modern American. Dr. Buller, back in Canada, proceeded to attack the horizontal distance record, formerly held by Miss Walker with a range of 14 feet. Dr. Buller set to work very scientifically, invoking the principles of ballistics, about which Miss Walker, being only a woman, could not be expected to know anything. Taking

advantage of the mathematical fact that the greatest horizontal range of a projectile is attained when the gun is set at an angle of about 45°, he set his guns at about that angle. Furthermore, he discharged them artificially by means of a method discovered by Pitra in 1870, which, however, adds nothing to the impetus of the projectile, and merely hastens the time of release, enabling the experimenter to avoid waiting an hour or more for the gun to discharge itself. Dr. Buller used a local Canadian strain of *Sphærobolus*. I may therefore claim his best result—a range of 18 feet 7 inches—for the Empah. He wrote in 1933: "Up to the present, 18 feet 7 inches is a world record for horizontal distance in fungus gunnery."

Dr. Buller has pointed out that, again according to the theory of ballistics, the horizontal range of a projectile should be in theory close to twice its vertical range; he therefore thinks that if the fungus-guns which gained the altitude record for Miss Walker had been tested out over a horizontal distance, a range of 20 feet should have been exceeded. However, facts are facts, and up to the time of going to press the honours in fungus gunnery stand as follows :

ALTITUDE RECORDS (VERTICAL RANGE)

1. Miss L. B. Walker (U S.A.)	14 feet 5 inches
2. Dr. Reginald Buller (Canada) .	about 7¼ feet

HORIZONTAL RANGE RECORDS

1. Dr. Reginald Buller (Canada) . . .	18 feet 7 inches
2. Miss L. B. Walker (U.S.A.) . . .	17 feet 3 inches

According to von Marilaun, an Austrian botanist quoted by Dr. Buller, 18 feet is a greater horizontal distance than that of which the seeds of *Viola canina*, *Dorycnium decumbens*, *Geranium columbinum*, and *G. palustre*—all of these being well known as seed-projecting plants—are expelled. Plants of the species *Lupinus digitatus* and

Acanthus mollis, and of the genera *Hura* and *Bauhinia*, expel their seeds to greater distances than *Sphærobolus* can shoot; the two latter plants having had ranges of 45 feet (14 metres) and 49–50 feet (15 metres) recorded respectively, *Lupinus digitatus* having about half that range. But such plants are relatively huge and have heavy seeds, and the comparison is not unfavourable to tiny *Sphærobolus*, the gun of which is not much more than a speck, and has a light projectile encountering relatively considerable resistance in air.

In the hope of winning new admirers for little *Sphærobolus*, and with a view to inciting fresh champions to enter the field, I may mention that species of *Sphærobolus* are widely distributed, and occur in Great Britain. There seems to be grave danger of the U.S.A. winning both the vertical and horizontal fungus-gunnery championships, if she once becomes aware of the possibilities of her material, while other nations do nothing with theirs. If America does scoop both records, it will be only fitting; has she not the biggest, widest, highest, and longest of everything else?

F

MICROBES AS SOURCES OF PERFUMES, DRUGS, DYES, AND FOOD

" AND died in the odour of sanctity." There is justification for believing that this phrase is something more than a metaphor, and that the " odour of sanctity " is a real odour. Dr. F. A. Hampton wrote in his engaging book on scent[1]: " It is possible that the sweetness of the first products of decomposition may play a part in the fragrance that has been recorded of the bodies of saints and anchorites immediately after death."

It is true that substances identical with and akin to one of the principal sweet-smelling constituents of otto of rose are produced in small amounts during the putrefaction of meat and other proteins. Ambergris is a form of decomposed cuttle-fish. However, in this section it is not my purpose to discuss the very numerous substances formed outside microbial cells. I shall include only those cases in which the actual microbe, or a simple extract of microbial tissue, is the material used by man.

Cell-material of at least two kinds of microbes contributes to the fashionable world's aura of sweet smells. One of them comes from a member of the actinomycetes, that class of lowly organism which, as regards complexity of structure, come between the bacteria and the true fungi. The other is derived from a lichen, the structure of which I have explained in Chapter II: it is a consortium of microscopic algæ with a degraded but recognizable fungus.

I have not hitherto had much to say about the actinomycetes, and, indeed, one of their chief claims for notice is

[1] *The Scent of Flowers and Leaves: Its Purpose and Relation to Man.* Dulau; London: 1925. 6*s*.

odour. It is said that actinomycetes are responsible for the earthy odour of soil, and it is a fact that many, though not all, of the actinomycetes do emit a peculiarly mouldy odour when they are grown on the usual laboratory media. Knowing this, a manufacturer of raw materials for the perfume industry asked the Rothamsted authorities to supply him with some strains of actinomycetes for cultivation on the semi-large scale, so that he could extract the odorous substance and sell it. He did select a few strains, and I believe that his product is on the market. The appearance in the Rothamsted report for 1934 of a reference to this application of soil microbes led to some sensational articles in the daily press, suggesting that perfumes were being made from soil. This was doubly a misapprehension; for one thing, no soil is used at any stage of the process after the actinomycetes have once been isolated; and, secondly, the product obtained from the actinomycete is not itself suitable for use as a perfume. Most people would say that its odour is unpleasant. But many materials which are unpleasant when concentrated are useful to the skilled perfumer for modifying the "note," as he calls it, of a blended perfume. The earthy-smelling substance derived from the soil actinomycete has a value: a trace of it in a perfume intended to suggest an autumn evening might convey that essential touch which means distinction! You will understand that soil actinomycetes do not provide a perfume ready-made.

The same remark is applicable also to the lichens used in modern perfumery for the production of *mousse de chêne*. This is a sirupy extract of the odorous principles of several kinds of lichens. The name is really a misnomer, for lichens are distinct from mosses, and trees of several species besides oaks furnish the *mousse de chêne* of commerce. *Mousse de chêne*, therefore, being a mixed product from several species of lichens growing on several species of trees, offers scope for discrimination to the buyer of per-

fumery raw materials. In addition to this sirupy extract, solid " concretes " and " absolutes," as they are known in the trade, are available; these are highly refined, and are correspondingly expensive. Whereas " concretes " and " absolutes " are used in only the most delicate kinds of perfumes, ordinary *mousse de chêne* is frequently a constituent of the cheaper and more robust types of perfume, such as those used in soap. Your handkerchief perfume may not have a lichen extract in it, but there is quite a good chance that your soap contains *mousse de chêne*, unless it is of the verbena or eau de cologne type. The rather heavy odour of *mousse de chêne* does not commend it as an ingredient of such lemony scents. *Mousse de chêne* is valued not only for its own sake, but because it has the property of " fixing " other odours. It assists in retaining more fugitive odours with which it is blended, and makes the complete perfume more lasting, while contributing thereto its own peculiar, not unpleasant, " arboreal " odour. The collecting of lichens and the production of *mousse de chêne* is organized on the large scale, and the product in its ordinary form is relatively cheap. The lichens are collected almost entirely in the Mediterranean region, especially French Morocco. Until recently, the " moss " was worked up almost exclusively in Grasse, but a factory for production of the extract has been established at Meknès.

Note that, although the odour of *mousse de chêne* is usually described as " woody," the lichens collected for the manufacture of *mousse de chêne* live independently of the trees; the lichens use the trees merely as supports. Therefore, the odour of the lichens owes nothing to constituents of the bark or wood.

Dried powdered lichens were used as perfume ingredients in former times; they were among the perfuming ingredients of hair-powders, in the days when those were fashionable.

A number of lichens have been used as sources of dyeing material by peasants and crofters; the Hebridean *cudbear*

is an example. Incidentally, it may be remarked that the odour of Harris tweed is often said to be " peaty," and to be due to peat smoke. The odour suggests that it may be due to a lichen, though I am not familiar with the processes undergone by Harris tweed, and cannot suggest how the lichen odour pervades Harris tweed irrespective of colour. Nowadays the use of lichen dyes is restricted to amateurs,[1] and to relatively few peasants and crofters. A species of lichen—not " Iceland moss," however—forms a large part of the pasturage of reindeer.

The celebrated colouring matter *litmus* is derived from a lichen. It is not a dye, because, as is well known, it changes colour very easily, being blue in alkaline conditions and red in acid, and for that reason has an honourable record of use in chemistry.

Only one microbe now figures in the official list of drugs in the British Pharmacopœia. That one drug is ergot. The term " ergot " is used indifferently for the fungus itself (*Claviceps purpurea*) and for the diseased grains to which it gives rise on cereals, notably wheat and rye. Ergot, however, is usually understood to mean the diseased grain; since this, in an advanced stage of the attack, is almost wholly composed of fungal tissues, the distinction is not significant, and when one reads that the export of ergot from Spain in a recent year was a hundred tons, more or less, the magnitude of this traffic in microbial tissue can be realized. The ergot (ergotized grain—usually rye) of drug commerce is compounded into an extract used in obstetrical cases. Sometimes refined preparations are used, involving only one or two of the physiologically active principles present in the diseased grain. Ergot has long been known as a disease of cereal grains. The consumption by human beings of partly ergotized grain has led to outbreaks of poisoning, affecting both sexes, and called ergotism.

The use of ergot as a drug is comparatively recent, and

[1] See books mentioned at end of this Section.

there is not even a very long tradition behind it. Its beneficial use apparently originated amongst Central European midwives in the seventeenth century, and knowledge of the drug spread only slowly. About the beginning of last century ergot (as drug) was introduced into the United States, from which its use spread to the rest of the civilized world.[1]

Although perhaps it is not strictly microbial, I should like to mention one of the curious examples of the uses of fungi mentioned by Dr. J. Ramsbottom.[2] He points out that very many fungi are used as native medicines in various parts of the world, and that special interest lies in study of the beliefs associated with well-known fungal species. The fungus *Cordyceps sinensis* " with its attached parasitized caterpillar " is sold in China in bundles tied up with red silk. Though Dr. Ramsbottom quotes Captain Kingdon Ward as saying that his men were always on the look-out for this fungus, he does not say for what ailment it is used. I have been told by a Chinese colleague (Mr. H. K. Chen) that it is sold chiefly in the sub-tropical parts of China, especially Kwantung. Further details about this fungus are given by R. T. Rolfe and F. W. Rolfe in *The Romance of the Fungus World* (Chapman & Hall, London, 1925). These authors give the Chinese name: *tong-chong-ha-cho*; I am privileged to add that this means: " winter-insect-summer-straw." The idea underlying this name appears to be that the doomed caterpillar becomes a plant in summer, after it has acquired its fungal vegetation !

Before leaving the subject of medicinal fungi, I should recall the case of yeast, which in dried form is the basis of several patent medicines. Yeast is alleged to be of benefit in some digestive disorders and to promote general well-being; moist cakes of a special strain of yeast are, or used

[1] G. Barger, *Ergot and ergotism.* Gurney and Jackson; Edinburgh: 1931. 15*s.*

[2] Pres. Address, *Brit Assoc. Report*, 1936, 189–218 [17977].

to be, extensively advertised in the United States for that purpose.

Such yeast might almost be considered to be food, and indeed yeasts have been proposed as food in times of scarcity; Dr. Ramsbottom gives some examples in his paper.

Apart from the yeasts, and fungi of the mushroom type, and such lichens as are used as food, only two kinds of microbes have been recorded as a food.

Mr. Chen has told me that a very delicate soup is made in parts of China from an alga—a species of *Nostoc*. He believes it comes from the sea, but is not certain. Marine algæ are used as food in many countries, but they are not usually microscopic, whereas this Chinese one in question is a true microbe, consisting of chains of minute green cells. It is known as *to-fa-tsai*, which means "hair vegetable." Mr. Chen tells me that the soup is regarded as a great delicacy, though, like the celebrated birds'-nest soup and other rare dishes esteemed by the Chinese, it is almost insipid. It appears that such costly preparations depend for the value less upon their flavour—which is almost absent—than for the "showing-off" of which they are the means. We thus have a microbe as an expression of Chinese *crânerie*.

A species of bacteria has also been used as food. The case of bacteria as food is made stranger by the occurrence of the esculent bacteria in substantially pure culture in soil. These edible masses of bacteria are known only in two restricted volcanic areas in Japan,[1] on the slopes of the volcano Mount Asama, and nearby. The bacteria are found a little way under the surface of the soil, and are known by a native name meaning "the boiled barley of Tengu," Tengu being the name of a deity. This name suggests both the aspect of this extraordinary food, and the esteem in which it is held by the few who have access to it.

[1] Y. Okada, *Soil Science*, 1937, **43**, 367 [20327].

BOOKS

Ethel Mairet's *Vegetable Dyes* (Faber & Faber; London, 1938, 5*s*.) contains a good list of references to other publications, but does not mention: O. A. Bériau (Directeur-Général des Arts Domestiques de la Province de Quebec), *La Teinturerie Domestique* (Quebec, 1933), which gives some recipes for dyeing with lichens.

There are no popular works dealing in detail with other microbiological subjects mentioned herein, but Dr. Ramsbottom's paper will probably be found readable by the non-specialist.

G

STOMACH SARCINAE

WHILST certain fermentation processes have been known from the beginning of the development of bacteriology as a science, it was given to *Beijerinck* to discover in 1905 a fermentation process which had remained unnoticed. In a paper published in that year[1] *Beijerinck* described an extremely interesting enrichment procedure, which with almost unfailing regularity brings to the fore a large sarcina-shaped micro-organism causing a vigorous fermentation in sugar-containing media, such as beer wort. The discovery of this quite unexpected fermentation was the result of a series of systematic experiments made (in part jointly with *Dr. N. Goslings*) to examine the question as to which are the organisms able to develop under anaerobic conditions under conditions of high acidity. In this investigation it was found that if the development of moulds and yeasts was suppressed by complete exclusion of air, the addition of somewhat higher amounts of inorganic acids to beer wort inoculated with garden soil almost invariably led to a fermentation which was marked by the development of large sarcina packets.

Now it happened that *Suringar*, professor of botany at the University of Leiden, who had been *Beijerinck's* teacher in his student period, had published a monograph on the remarkable sarcina noted by *Goodsir*, a Scottish physician, as long ago as 1842.

Goodsir had observed the occurrence of regularly formed packets in the stomach contents of a patient, and had described these formations under the name of *Sarcina ventriculi*. This observation was repeated from time to

[1] *Versl. Kon. Akad. v. Wet. Amsterdam*, 1905, **7**, 580 [22425].

time by medical investigators, who encountered the organism especially in cases of *stenosis œsophagi.* It was soon suspected that a close connexion might exist between the presence of the sarcinae and a gas-development sometimes occurring in the human stomach. However, no proof for the correctness of this assumption could be furnished, because it appeared impossible to cultivate the organism *in vitro. Suringar* was the first to prove the vegetable nature of the organism, and, from this time on, it has been ranked with the bacteria.

There is no doubt that *Beijerinck* was thoroughly acquainted with the organism to which his former teacher had once devoted so much of his attention. It is, therefore, not surprising that *Beijerinck* should have taken into consideration, in his first paper, the possible identity of his new fermentation organism and *Goodsir's Sarcina ventriculi.* It should, however, be realized how daring a thought this was. On the one hand, an organism which appeared, on the evidence of enrichment cultures, to be practically ubiquitous in the soil; on the other hand, a medical " living curiosity " which nobody had ever seen develop outside the human body.

Beijerinck's studies of his new fermentation organism had made him familiar with one especially remarkable property, *viz.* that the cultures could only be transferred into fresh media as long as the fermentation was still active. Obviously the bacterium dies off very quickly after fermentation ceases, partly because as a strict anaerobe it cannot withstand traces of oxygen diffusing into the medium, partly perhaps owing to the action of the organic acids formed in the fermentation.

This observation made *Beijerinck* realize that a cultivation of the stomach sarcina *in vitro* would only succeed if the stomach contents in which it was present were transferred immediately after their collection into a medium permitting optimal development. Neglect of this point might well be

responsible for the failure of earlier investigators to cultivate the organism.

It was not until six years later that *Beijerinck* got the opportunity to submit his hypothesis to an experimental test.[1] This test led to a completely satisfactory result. The flasks of beer wort inoculated with the fresh stomach contents of a patient entered quickly into a strong fermentation, and this culture could be transferred in exactly the same way as the soil organism. In other respects, also, complete identity of the two organisms was established.

The excellent monograph which *Beijerinck's* collaborator *Smit* in recent years has devoted to *Sarcina ventriculi* and some related organisms, throws a clear light on the remarkable properties of the representatives of this group.[2] *Smit* shows that the wide distribution of *Sarcina ventriculi* in nature seems quite opposed to the extreme sensitivity of the organism when cultivated in pure culture. A resolution of this apparent paradox has not yet been reached. Further work on this subject seems most desirable, and may be of great importance for our general insight into the conditions which determine the survival of microbes in nature.

This Section was written by Professor A. J. Kluyver, Beijerinck's successor in the Chair of Microbiology, at the Technische Hoogeschool, Delft (Holland). He wrote this Section as part of an appreciation of Beijerinck's scientific work. The complete essay will appear shortly in a book entitled: *Martinus Willem Beijerinck, his Life and his Work*; edited by Dr. G. van Iterson Jr., Dr. L. E. den Dooren de Jong, and Dr. A. J. Kluyver. It will be published by Messrs. Martinus Nijhoff N.V., The Hague. This biography will also appear as an addendum to Vol. VI of Beijerinck's *Collected Works* (*Verzamelde Geschriften*), the first five volumes of which have already appeared.

I gladly acknowledge the courtesy of Professor Kluyver and Messrs. Martinus Nijhoff in giving permission for the publication of this extract from their forthcoming works.

You have read the Section exactly as Dr. Kluyver wrote it, except for the omission of an introductory sentence and a final paragraph. Having started this book with no knowledge of microbiology at all,

[1] *Versl. Kon. Akad v. Wet. Amsterdam*, 1911, **13**, 1237 [22425].
[2] Jan Smit, *Die Gärungssarcinen*, eine Monographie. Jena, 1930.

you will appreciate that there is significance in the fact that you have read, and tolerably well understood, a learned exposition written by a microbiologist for microbiologists, and presented to you without comment and without any simplification of style or treatment.

Now I will give Profesor Kluyver's final paragraph, and a comment or two, leaving you to decide whether you enjoy microbiological thought sufficiently well to want to enter further into its implications:

Finally, it seems probable that the recent procedures for the preparation and preservation of silage, based on the reputed absence of microbial life under anaerobic conditions as soon as the acidity of the medium corresponds to *p*H 4·0 or lower, will before long lead also to the realization of the great practical significance of the fermentation process discovered by Beijerinck.[1]

[1] Smit's experiments have shown definitely that development of *Sarcina ventriculi* is possible in media having a *p*H only slightly above 1·1. [*p*H is a measure of acidity. Silage is a form of cattle food prepared by fermentation (see Chap. XIII, "Bad to be Good"), and is nearly as acid as vinegar is, but not as acid as the gastric juice of the human stomach. The meaning is, that Smit has found that *Sarcina* can tolerate conditions rather more acid than those of the normal gastric juice. This is reasonable, for otherwise the sarcinae in the stomach would be living "on the edge of existence."

Martinus W. Beijerinck (1851–1931) was a native of Amsterdam and an exceedingly ingenious microbiologist, though his methods of doing what nobody had been able to do for sixty years sound simple, now you know what they are. He used beer wort to which had been added enough hydrochloric acid to make it as acid as gastric juice is; this required an amount of acid small in itself, but relatively much larger than anyone had thought of using in bacteriological media.

Beijerinck was the discoverer (in 1901) of *Azotobacter chroöcoccum* and of *A. agilis*; he found the latter in the Delft canals, and the former almost everywhere around Delft in the canals and in "fertile garden soil." *Azotobacter chroococcum* has since been reported in fertile soils all over the world. For details of this remarkable man's life and work I must refer you to the books already mentioned. I wish I had space to give a biographical sketch of this irascible, sensitive, and lonely man, whose skill in coaxing microbes to manifest life was so very much greater than his understanding of people, and to whom you and I owe so much.]

EPILOGUE

Opening with a kindly warning to prospective examinees, and proceeding to treat of matters affecting a wider audience

The liberation of the human intellect must, however, remain incomplete so long as it is free only to work out the consequences of a prescribed body of dogmatic data, and is denied the access to unsuspected truths, which only direct observation can give.

R. A. Fisher: "The Design of Experiments."[1]

MR. GEORGE BERNARD SHAW has approached the subject of fascism and democracy from another angle in *The Intelligent Woman's Guide*. He has also broadcast a talk on Education, in which he possibly exaggerated the conservatism of examiners—though there is not much doubt that they are conservative where factual knowledge is concerned. Military Staff Colleges, for example, are said to be always "preparing for the last war."

Before Mr. Shaw came to the microphone with his warning, my colleagues and I had been in the habit of saying something less witty, but rather like it in effect, to parties of young biologists visiting Rothamsted Experimental Station. We told the eager young students that it was doubtful whether our information would be of use in their examination answers, because a large part of what we had to say was so new that the examiners wouldn't have heard of it. And, what an examiner has not heard of, is not knowledge. We got some fun out of this at times, when the prospective examiner was also present and smiled wryly.

I welcome this opportunity of giving to a wide audience the substance of our fifteen-minute talks on soil bacterio-

[1] Oliver & Boyd; Edinburgh: 1937. 12*s.* 6*d.*

logy: you will find it, a little enlarged, on pages 141–149. The rest of the book is embroidery on the same theme.

However, as I have said, for a time you must be cautious about using the information, especially that referring to soil bacteriology. The information is as correct as it can be made within the limits of the book, but there is a risk that it will be marked with the examiner's equivalent of "wrong" if you use it. Fortunately for you, this is a "Pelican" book, and it is possible that the examiners also will have read it.

In the larger world, facts in themselves are less important in reaching a result. Life consists of behaviour in relation to facts, many of which are produced by scientists. Living cannot be happy unless it is in adjustable harmony with the material world. Because science is producing facts and things (such as motor-cars) at a greater rate than man's adjustment to them, it is often said that science is a disruptive factor of life, and that we should be happier without it. The people who say that sort of thing also believe that science is wholly an affair of the laboratory. I shall show that both these beliefs are wrong. They arise because the methods that *produce* inventions have not yet been applied to the *utilization* of such things. The motor-car engine is the scientific result of much laboratory work and bench-testing, but no sort of scientific principle governs the use of the motor-car on the road. The use of the motor-car is subject to only arbitrary regulations.

Most of this chapter will be wasted on you unless you understand the significance of Mr. G. K. Chesterton's remark that the electric signs on Broadway are a beautiful sight for anyone who cannot read.

The effects of vehicles on roads has been the subject of much investigation, but the more fundamental question of the correct relationship between rail and road has never been scientifically enquired into. We have the farce of engineers trying very hard to produce a road on which motor-

vehicles cannot skid, even while railways exist as a demonstration of how lateral skidding may be entirely prevented. Consideration of railway practice is held to be outside the province of road engineers. While road engineers are expected to apply science to one set of things (and railway engineers are expected to use science in their set of things), the greater questions affecting all of us go untouched by scientific thought. Science has hitherto been applied to problems of the production and use of comparatively small articles, and it has not been realized that science can be applied to problems outside the laboratory and workshop. In fact, almost the only suggestion that has been made for the enlargement of the scope of science has been the suggestion that more use might be made of scientists in administration and government.

Now, even scientists themselves are not whole-heartedly in favour of the latter idea. They are aware that scientists, when given executive posts, have often not become good administrators. This reluctance is due to an opinion only; it may not, after all, be a correct opinion. A more cogent argument is this: that scientists, being men and women, are only men and women after all, and if they were called upon to make *ex hypothesi* some political or social decision, the rest of us would have no surety that it would be a wiser decision than one made in similar circumstances by a politician untrained in science. The scientists' decision might well be the worse.

The contribution of the scientist to social life is not himself or his opinion, but his method. So long as scientific methods were—as in Pasteur's day and until quite recently—methods that could not be satisfactorily applied outside the laboratory or experimental farm, so long was the scientist compelled to deduce only from test-tubes or machines or experimental animals. His reputation as an impractical sort of person of bounded vision remains and will remain for some time, until it is realized that the

methods of science are *now* capable of application to many social problems. That became possible because scientists have at last investigated the methods of experimenting, and have submitted the abstract technique of experimentation to as close and impersonal a scrutiny as they formerly gave to the study of concrete things such as chemicals and bacteria. It is this new and better technique of making experiments that has opened up the possibility of obtaining valid answers from social experiments. Note the *valid.*

But I must explain my terms before I go any further, or you are almost sure to misapprehend me, just because the word "experiment" probably does not connote the same thing to you as it does to me. You have been doing, or reading about, experiments all through the book, and I have only in one place suggested that the term "experiment" might perhaps not be fitting. The word, unfortunately, has several meanings, and the idea usually conjured up by the phrase "social experiment" is quite different from that implied in any scientific experiment, old style or new. You may read that "Russia is a vast social experiment." Whether Russia (*i.e.* the Russian style of government) has succeeded or not, is a matter of individual opinion. As an experiment, Russia fails to give any valid, because objective, answers. So it is not an experiment in the modern sense. It might be called a demonstration, but of what—that Russia is now Russia?

It might as well be said that Manchester is an experiment (in gathering together a large number of people mainly dependent on one industry); or that Edinburgh is an experiment on having a capital city dependent on professional and regional services rather than on industry. Such a use of the word seems strange. Now see how it reads if I say that "A new bungalow town has been laid out as an experiment." The word seems to be in its right place again. Isn't it because the word "experiment" is so often a synonym for "innovation"? Say: "Russia is a vast

social innovation," and the sense has not been altered a bit.

Clearly, in talking about experiments and experimenting, we must be careful to be at one as to meanings. It is all the more necessary, since the new technique of experimentation —that which can now be applied to many social problems— involves the use of that branch of science known as statistical analysis—which has nothing in common with " statistics " in the ordinary sense (of data about imports, acreage, etc.). For me to try to re-orient your ideas of these two words would take me far beyond the limits of this book; therefore I will merely say that whereas " anything can be shown by statistics " (old saying, using old sense of the word), the modern science of statistics is designed to test hypotheses mathematically by means of data obtained by sampling or experiment. The modern statistics, applied to a set of data, gives an exact measure of how often a conclusion drawn from that data will be wrong. We can therefore ascertain that, for example, it is likely to be wrong once in twenty times. Statistics also estimate relevant quantities, and their margin of error, in a properly designed experiment. Far from making it possible to extract conclusions to suit various tastes from one set of facts, the modern statistics is impersonal, inexorable. In the hands of a democracy willing to probe for better things, it could be a tremendously powerful blade.

By now you may be thinking that I have got a long way from microbiology, and that the foregoing has nothing to do with the book. But, you will have absorbed some scientific notions (as distinct from facts about microbes), and they have laid the bare foundation of an appreciation of the newer experimentation, for which I have just made a very big claim.

In the eighteenth century the word " experiment " usually meant a trial to see whether a thing could or could not be done. It is still so used; that is, in the sense of

demonstration. In scientific circles it has acquired a more precise meaning bound up with ideas of enumeration and quantitative measurement, while, in popular language, as we have seen, it has been degraded to an indefinite sense, which can now be dismissed.

The modern sense of "experiment" implies certain concepts, of which some have already been made use of in the experiments put before you in the book.

The idea of "asking only one question of Nature at one time" is dead. It is dead, not because we are now *enabled* to ask several questions at a time; it is dead because we *must* examine all the factors, if we are to get trustworthy results from any but the simplest comparisons. Time itself (or its consequences) is often an important but unrecognized factor. One question is dependent on others. If we want to find out how to get the biggest yield of wheat from the use of fertilizers, we have to recognize that we cannot find the most suitable fertilizer without doing the test on some variety, and that we cannot find the highest-yielding variety without using fertilizers to attain the maximum yield of which each variety is capable.[1] Hence we have to combine a test of all likely combinations of fertilizers with all promising varieties of the wheat: tests of each factor one at a time are of no use. Some simplification is necessary, but to over-simplify is to reduce the value of conclusions.

Factorial experimentation, which is valid, has killed an old idea, that never was or could be valid in complex cases—which most social problems are. Only modern statistical analysis can give us *every time* the assurance of being substantially right about existing things capable of being measured; and only factorial experimentation can provide the same exact assurance about the value of new developments.

[1] F. Yates, "The design and analysis of factorial experiments." *Technical Communication No.* 35, Imperial Bureau of Soil Science; Harpenden: 1937. 5*s*.

EPILOGUE

Already it has become unthinkable in agricultural science that anyone wishing to find out, say, the best fertilizer for a given purpose, should rely on the equivalent of sworn testimony, backed by the opinion of no matter what expert, derived from a survey of fields on which the material happens to have been used: what I may call the "Royal Commission" procedure has already been discarded by agricultural science in favour of the experimental probe.

The difficulty of applying science to social problems is not so much to design sound experiments as to get the public into a readiness to accept the ideas underlying such experimentation. The principles of experimentation are the same whether the experiments are performed on bacteria, or on textiles (to take a very different material). They are not essentially modified if applied to determine, say, whether narrow roads are more dangerous than wide ones, subject to the proviso that for such an enquiry no special experimental roads need be built; existing ones could probably be "grouped" so as to provide material for analysis on modern lines: the actual varying densities of traffic of housing; degrees of proximity to schools; and so on, would afford what I have called "treatments" (the statistician calls them "factors": hence "factorial analysis").

In the last example the test can be made by existing agencies, without requiring the co-operation or even the knowledge of the public. The conclusions can also be implemented fairly readily.

Suppose, however, that the results of such an analysis of road factors were implemented, by the building of new roads in accordance with statistically sound conclusions about roads. Would such a consummation be desirable? It would not, because something has been forgotten. We cannot have roads as roads—*in vacuo*, as it were.

Roads, perfect or not, are collectively only one factor in a larger scheme of things. They cannot socially be considered without railways and canals; the two latter cannot

be considered except in relation to agricultural drainage, urban water-supply, and so on. If we apply science to society, we are led irresistibly to a scheme of planning. " An integrative view " was the phrase I used in speaking of microbes.

The analyses given in Chapter VII have shown which of the conclusions are valid, and which only seem so. This is a tremendous advance over " consideration of the available facts," " decisions taken in the light of reports," and other stock phrases of Royal Commissions, board-meetings, and committee-rooms. I hope you see why I have faith in the potential benefits of the application of science to problems outside the laboratory.

The idea that authoritative pronouncements have a great value is so widespread, that it is worth enquiring how far an authority can legitimately go in speaking authoritatively. The police officer, for example, may have a good card index or an excellent memory; he is therefore entitled to say that drunkenness has decreased (or increased) since 1912, and that there are fewer accidents (or more) by night than by day. These are facts, and to the extent that he is able to produce facts about his district he is an authority.

I am totally unable to say from mere inspection of the data given on p. 94 whether the increase from phosphate and lime was significant or not—*i.e.* had any value as a basis for action. If pressed to draw a conclusion, nobody could do better than plump: experience of phosphate and lime would have no value, and " expert " or any other opinion (honest or biased) would be worth about as much as the toss of a coin. My experience helps me *now* only because I am aware of the possibilities of statistical analysis, and I am *now* expert enough to refrain from deciding until an analysis has been performed. But, twenty years ago, nobody had that tool.

The value of authoritative opinion depends only sometimes on accumulated experience. It depends in part on

the respect in which the "authority" is held, and on the actual or supposed honesty of the giver. Its weakness is that its conclusions can only be personal, and may be biased. Statistical conclusions are inexorable, and are capable of being reached by any suitably equipped person.

In public matters there is often a good deal of invalid reasoning from the results of a trial not capable of giving a determinate conclusion. Thus it might be said that the presence of tramways is dangerous in a road, it having been noted that accidents are high in certain streets in which tramways run. The further conclusion is then drawn that the removal of the tramways is desirable from the point of view of safety. Neither of these conclusions necessarily follow from the data, nor does the second follow from the first. It is likely that most of the roads in which tramways run would be dangerous anyhow (given traffic of our present types), being urban roads of high density of use. If the removal of tramways is followed by a decreased number of accidents, that does not prove that their removal is justified.

The sought-for comparison does not exist—the new factor of *time* having been introduced. Time may have brought about a change in other traffic (*e.g.* an increase in the efficiency of motor-car brakes). The removal of some other class of traffic instead of the tramways might have brought about an equal or greater reduction in the number of accidents.

The "experiment" shows no more than the fact that accidents have become fewer after a certain time (if that is a fact); it cannot help in deciding which of the many factors involved have produced the result. Any other conclusions drawn from it are not only invalid, but do not give the public the assurance that the best thing has been done to decrease accidents. This assurance will emerge from a properly designed experiment.

Unless valid tests are made, the opinions of a Chief

Constable or other heavy-weight "authority" have no determinable validity. Neglecting to consider the interaction of factors is the chief error underlying authoritative pronouncements on matters capable of being, though not submitted to statistical analysis. Until recently, there has been no test by which the validity of such pronouncements could be judged.

In 1923, Cutler, Crump, and Sandon [1] showed that in Rothamsted soil the numbers of bacteria, determined by the plating method, underwent striking variations from day to day. This result was accepted with difficulty by Smith and Worden of Washington D.C., who, therefore, did a number of experiments. They concluded that American soil bacteria behaved more soberly than we thought ours did, and did not show such fluctuations in number. The data published by Smith and Worden were re-examined here, and it was proved that the fluctuations in Smith and Worden's soil were as real as those found at Rothamsted. Two honest and careful observers had unwittingly come to a conclusion opposite to that warranted by their observations! This was one of the first victories of the new methods of examination of facts. It had "become possible to make a decisive test of the reality of day-to-day changes" in Smith and Worden's particular set of counts. Ten years earlier, Smith and Worden's conclusions would have been unhesitatingly accepted as valid, for lack of any test to judge them by.

When a possible or actual conclusion does not suit powerful interests, only public education can ensure that a proper enquiry is not burked, and that no valid conclusion is suppressed, twisted, or given bias. It is, however, something to know that the tool exists, for then—or I should say, *now*—you can watch for it to be used in your collective

[1] D. W. Cutler, Lettice M. Crump, and H. Sandon, *Phil. Trans. Roy. Soc. Lond., B*, 1922, **211**, 317 [16192]; N. R. Smith and S. Worden, *Journ. Agric. Res.*, 1925, **31**, 501 [10965]; H. G. Thornton and R. A. Fisher, *Soil Sci.*, 1927, **23**, 253 [20327].

interests. Since the test now exists, I submit that omission to use it is a grave handicap to city or state.

The new principles of experimentation at which I have hinted are almost wholly a British development. They are so new that most scientists, even, are still unaware of their possibilities. Only fifteen years ago the knowledge did not exist. It remains to be seen whether the new principles will be applied for the betterment of social conditions. If they are not applied, our condition will be no better than the adoption of dogma, to challenge which is treason. The latter condition is Fascism, by whatever name it be known.

Mr. H. G. Wells has suggested the compilation of a *World Encyclopædia*, to contain all available knowledge as a basis of social conduct. If such an encyclopædia consisted solely of facts, what guarantee is there that the facts might not be " coloured " ? Truth will not necessarily prevail, but at least it must first be decided what truth is, before " true facts " are admitted into the encyclopædia. If truth about the use of fertilizers were required, it could be obtained from Rothamsted or some similar institution, because agricultural experiment stations are remarkably free from bias, and are deaf to commercial cajolery. But, if truth were sought about the merits of different forms of transport, or any other social service or supply having a commercial nexus, there exists no correspondingly independent organization enjoying the position that Rothamsted does in its sphere. It would be necessary to go to a host of financially interested, non-experimental sources, who might be silent or worse about modes or things that happened not to fit in with their views or systems. For example, the railway companies might be very " discreet " about the mono-rail. Possibly the only surviving facts would be found in the possession of an amateur.[1]

[1] The assembling of facts about local government depended upon the accident that Beatrice and Sidney Webb (now Lady and Lord Passfield) had both the inclination and the means to perform it.

It is often said that we need facts, not opinions. This is true in so far as it is rare for each person's opinion to have the same value. One of these rare examples is given in Chapter II. In music and art, opinion is all that matters. Elsewhere, I am inclined to suspect the value of any person's opinion, except about matters on which no experimental test has been or *can be* made on modern lines.

A static mass of unrelated facts will not help us. We need valid conclusions, which can be obtained only by experimentation and proper analysis, from facts as they emerge during the growth of knowledge. Agriculture is unique among industries in having independent, uncommercial centres of experiment.[1] Their existence is a pointer, but it will be nothing more than a pointer until popular education in scientific thought is more advanced. Mr. Wells's idea was put forward in 1936, but is nevertheless a pre-Fisherian concept.[2]

The value of science in everyday life does not depend upon a knowledge of facts about chemicals, mosquitoes, mining, microbes, or anything else. The facts can well be left for the specialist.

Discussions on the place of science in school time-tables have tended to overlook the fact that a scientific viewpoint is independent of the material studied. In the past there has been some justification for claiming that an "exact" science, such as chemistry, provided better training in critical thought than did anatomy or other of the descriptive sciences comprised in biology as then understood. This claim is no longer valid, for properly-taught elementary

[1] It is also unique in having the Imperial Bureaux of Agricultural Science, which are Mr. Wells's idea come alive so far as agricultural science is concerned.

[2] Dr. R. A. Fisher, F.R.S., Galton Professor at University College, London, and formerly of Rothamsted Experimental Station, has done more than any other man to found the new technique of experimentation. He is undoubtedly one of the greatest thinkers of the age.

biology can be made to provide as much mental exercise as any other science can.

The value of a scientific training to the non-specialist lies mainly in the acquisition of a critical faculty, accustomed to take no observable phenomenon upon trust, and to examine the basis for behaviour and beliefs. With these go necessarily the appreciation of certain fundamental concepts such as ratios, approximations, and the requisites of valid comparisons.

The time is not yet come for the scholastic teaching of the factorial mode of looking at social problems. It is, however, patent that a grasp of scientific concepts more elementary than some of those outlined in this book would profoundly affect a genuine democracy, if there be any such state. I shall conclude illustratively with a few everyday examples of that simple concept, a ratio. An experiment comparing two things often aims at expressing one number in terms of another, such as " treatment " result as a ratio of " control " result. A percentage is nothing but a ratio.

A ratio is meaningless unless both of its terms are known. The scientist so well appreciates this that he takes it for granted. High finance does not—openly. The question of the gold standard, for example, is a question whether a certain ratio shall be maintained between the cost of gold (which is unknowable, except in terms of goods) and the cost of goods, which is unknowable, except in terms of go d or some other arbitrary standard. I think that future historians will begin their accounts of this scholiastic dispute, not by saying " In the then state of economic theory . . ." but: " In the absence of any widespread grasp of the absurdity of trying to maintain a ratio between two unknowns . . ."

You may have noted that those countries which have little or no gold are not the worst off as regards unemployment. I do not suggest that the abolition of gold is a cure for unemployment—so many other factors being in-

volved. I do suggest, however, that having insufficient gold to " work " the gold standard has stimulated thought and has led to fresh action unhampered by notions built round a chimerical ratio.

Many hard-headed business men are taken in by the sophistry that a dividend is low because it is expressed as a small percentage. Five or six per cent. appears to them a reasonable dividend, because it is 5 or 6 in 100. But unless the capital is fixed and known, the 5 or 6 becomes meaningless (as it is intended to be) because no statement is made about what the 100 is equivalent to at any given time. The " watering " of capital consists of varying one term of a ratio, while hiding as far as possible the fact of its having been varied. It is a transparent device, but is still very successful.

A dividend may be looked upon as a percentage upon receipts as well as a percentage upon capital. The former is sometimes the fairer, because capital may be low if profits have been " ploughed back " into the business. On the other hand, the capital may be artificially swollen.

A problem occupying the minds of many people just now is whether the London Passenger Transport Board will be able indefinitely to pay $4\frac{1}{2}$ per cent. on a part of its stock (" C " stock). This does not sound a very high rate of interest, but, for reasons similar to those just mentioned, it is useless to argue about that. Four and a half per cent. would become $2\frac{1}{4}$ per cent. if the capital was doubled, or 9 per cent. if the capital were half of what it is. Unless the capital has always been what it now is, it would seem vain to try to maintain a ratio which, after all, is arbitrary and artificial. If asked to discuss whether the dividend should be maintained, a scientist would suggest an enquiry into past capitalization; he would also point out that any dividend could be maintained at any desired level just as effectively by altering capital as by allocating profits (supposing a sufficient excess of them). If the ratio is to

remain fixed, the scientist would not see anything immutable about either term of it. Financial authorities, however, do not take this detached view, and oddly prefer to regard one term of the ratio (the declared percentage of profit) as almost sacred.

There are in the London area thousands of people whose travelling expenses amount to six shillings per week. In order to approach a dividend of 4½ per cent. on its "C" stock, the L.P.T.B. has to have a ratio of working expenses to receipts of about 84 to 100. That is to say, 16 (not 4½) per cent. of the receipts is paid out in dividends on all stocks. That is nearly a sixth of the receipts, which are almost entirely composed of fares. Suppose that instead of aiming at fixed dividends on the Board's present capital, the capital were halved, and the inviolable percentages of dividend retained. Those who, in paying out six shillings weekly in fares, now pay nearly one whole shilling to the stockholders, would have to pay only about sixpence a week to those recipients. Thus, an incompletely-comprehended ratio or two may mean bread and butter, especially to those in search of work, who often have to give the preference to paying for transport over paying for food.

Science could be enormously human. Its implications extend far beyond the microbe world. I suggest that the extent to which scientific method is allowed to be applied to public affairs will be a measure of our efficiency as a democracy, and an index to freedom in the highest sense.

Britons pride themselves on their traditions of freedom. The British system of trial at law is generally supposed to be one of the most searching and least partial in the world. That other British growth, Parliament, seems to feel that it is outmoded in an age when mere opinion, in a world of new facts, become less and less securely based. Could we base executive decisions upon a system as fair as, but less fallible than, our system of trial at law?

GLOSSARY AND AMMUNITION

" *The question is,*" *said Alice,* " *whether you* can *make words mean so* ıany *different things.*"

" *The question is,*" *said Humpty Dumpty,* " *which is to be Master—* hat's *all.*"

" Through the Looking-glass."

NOTE: *n,* noun; *a,* adjective; *adv.,* adverbial.

Aerobe, n (aerobic, a). A common class-name for micro-ɔrganisms said to prefer to grow in presence of air, the air ɔeing assumed to a source of oxygen only. A synonym is *ıerophil. Oxygenophil* would be a term better than either, n the intended sense. See *Anaerobe.*

Anaerobe, n. Usually means a class of micro-organism ıble to live without more than traces of free (atmospheric) ɔxygen, and deriving its oxygen from that chemically ɔombined in sugars, nitrate, etc. *Anaerobe* and *aerobe* are egacies from medical bacteriology, which assumed that for nicrobes air was solely a source of oxygen. The term has ɔeen illogical since the discovery by S. Winogradsky in 1893 of a soil bacterium able to fix nitrogen without relying ıpon atmospheric oxygen. This organism naturally relies ıpon atmospheric nitrogen; it consequently does not live *ınaerobically,* as it is usually said to do. I have been ɔointing this out in private for some years, and my col-eagues have been mildly amused by what they thought was a ɔedantic distinction. In view of very recent work on biological nitrogen-fixation, the distinction seems likely to ɔe of real importance. (See also the *oligo*-words.)

Antisepsis, n; Antiseptic, a. See *Asepsis.*

Antiseptic, n. See *Disinfectant.*

Asepsis, n; aseptic, a. Implying the absence of sepsis (*i.e.* of suppuration, pus-formation: a bodily reaction of defence evoked by the presence of microbes, especially

bacteria, in a wound). A surgical term borrowed by laboratory bacteriologists, denoting (*n*) the condition in which bacteria are excluded, in distinction to *antisepsis*, which denotes a condition in which existing bacteria are killed by chemical or other means.

Calefacient, *a.* Heat-producing; producing heat. A special word has its uses, and I think this is in its right place on p. 69.

Ciliate, *n.* A class of protozoa possessing a large number of very short, rapidly moving " hairs " (*cilia*), whereas the *flagellates* (*q.v.*) have a small number of long hair-like appendages.

Coliform, *a.* Resembling in general characteristics *Bacterium* (*Escherichia*) *coli*, a rod-like bacterium which is the principal inhabitant of the normal human intestine. Consequently it grows best at about 37° C. (blood heat). Soil bacteria divide most quickly at temperatures around 20–25° C. Those are the temperatures to which the two groups are respectively accustomed.

Cresol, *n.* One of three organic chemicals similar to but slightly more complex than phenol, all four being compounds theoretically derived from benzene, and occurring in coal tar. In disinfectant work, phenol, the cresols, and other related compounds are classed collectively as " phenols."

Culture, *n.* A growth of microbes. Usually intended in the sense of a growth made and maintained in the laboratory.

Disinfectant, *n* and *a.* *Disinfectant* and *antiseptic* are words used to mean substances capable of killing or at least inhibiting the growth of microbes, especially bacteria. Lister's original " antiseptic " (carbolic acid, phenol) is now classed as a disinfectant, but it does not now seem useful to draw a distinction between the two classes. Disinfectants and antiseptics are not lethal to microbes below a certain concentration dependent on the substance and on the kind of microbe.

Flagella, n. Small whip-like formations attached to the cells of some bacteria and to those protozoa belonging to the class of flagellates. The flagella of bacteria and *flagellate* protozoa are different in kind.

Flagellate. When *n*, a class of protozoa possessing *flagella*; also used as *a*, to denote "possessing flagella" and a synonym of *flagellated.*

Genera, n (plural of *genus*). A group including sometimes one, but often two or more, *species* having some important characters in common. The genus *Azotobacter* contains relatively large disc-shaped bacteria (about 4μ diameter) able to fix nitrogen only aerobically. The species *A[zotobacter] agilis* has flagella and is consequently motile, while *A. chroöcoccum* has no flagella. In spite of this outstanding difference and minor characteristics such as appearance, and the ability to grow well on mannitol (*chroöcoccum* can, while *agilis* cannot), these and other species are sufficiently alike to be included in the genus *Azotobacter.*

In vitro (*adv. phrase*). Literally "in glass," meaning: under laboratory conditions as distinct from what happens *in vivo* (of animal or plant) or otherwise in nature. Use of the expression in microbiology implies a caution that the behaviour of an organism in nature may be different.

Macrobe, n. A large organism.

Medium, n (plural, *media*). A material (usually a mixture of substances) artificial or natural (*e.g.* a steamed potato) used for growing microbes; may be liquid or solid.

Mixed culture. Living microbes of two or more species (kinds) growing together in or on one medium, whether intentionally or not, so far as the microbiologist is concerned.

Nodule, n. In soil bacteriology, a small tumour-like growth formed on plant roots after infection by a microbe. Usually refers to those produced on legumes and which

contain, and were caused by, symbiotic nitrogen-fixing bacteria.

Oligonitrophilous, a (*oligo*: little). A word invented by Beijerinck in 1901 to connote the ability of nitrogen-fixing microbes to grow without much combined nitrogen, as they can get what they require from the air. This, and *oligo-aerophil* to denote the earlier *anaerobe*, represent awkward attempts to dodge the difficulties discussed under *Aerobe* and *Anaerobe*. Beijerinck, however, has fallen with "oligonitrophil" into a pit which is as disabling as *aerobe* is: nitrogen-fixing microbes require as much nitrogen as do those that do not fix nitrogen, only, the nitrogen-fixers do not essentially need chemically-combined nitrogen. They combine their own. Hence they are not *oligophilous* of nitrogen—only of combined nitrogen. The right set of words for these needs has not yet been invented. In the text the classical terms have been used in the usual sense; there has been no discussion of "anaerobic" nitrogen-fixing organisms.

Optimal, a; *optimum, n* and *a*. Used of "the best" condition for a given activity. The last part of the explanation given under *Coliform* may be rephrased thus: "The optimal temperature of growth of the coliform organisms is about 37° C. (blood heat), while the optimum for growth and activities of most soil organisms is about 20–25° C."

Pabulum, n (plural, *pabula*). Food, in a somewhat special sense. To speak of the "food" of bacteria sounds a little odd; also what is microbial food may not be human food.

Parasitism, n. The state of one organism living at the expense of another.

Protozoön, n (plural, *protozoa*). Literally, *primitive animal*.

Pure Culture. Living microbes of only one species.

Saprophytism, n. The state of a micro-organism living on matter that has died (plant or animal remains), or has

been thrown off by an animal or plant. Such a microbe is called a *saprophyte.*

Species, n (sing. and plural). As biologists are likely to argue for ever about "What is a species?" I can only suggest that in microbiology a species means the *kind* of microbe (in the narrowest sense of *kind*, not a class or a group). Each species is distinguished from other species by sufficiently outstanding characteristics.

Statistics, n (singular). Originally meant collectively "things of state" (of chancelleries, etc.), hence an easy transition to meaning facts about population, mortality, imports, acreage, and so on. Now used also to mean the branch of mathematics which is used for examining such crude data (or rather, variation in such data) for the purpose of drawing conclusions therefrom. The science of statistics is already a study demanding as high a degree of specialization as any other branch of science.

Strain, n. Variety, race, or line: a subdivision of a species. The Norfolk strain of *A. agilis* was closely similar to, though not necessarily identical with, Dutch strains of the classical species (p. 189). Some preferable strains of nodule bacteria confer more nitrogen on their host plant than other strains do. I give the French here because it is difficult to find in ordinary dictionaries: *une lignée.*

Substrate, n. Same as *medium*, but often with a faint distinction in the sense of a solid upon which microbes grow.

Symbiosis, n. The state of organisms of usually dissimilar classes while living together in helpful partnership. Often one of the partners becomes parasitic. A symbiotic organism is sometimes called a *symbiont.*

Thermophil, n; thermophile, thermophilic, a. A microbe that grows well at, or can tolerate, high temperatures. Some microbes exist in springs at a temperature of 70° C. (160° F.), and a few kinds of microbes in hot haystacks and other fermenting masses are supposed to endure temperatures up

to at least that height. The term *thermophil* is convenient to denote a class of microbes able to withstand higher than ordinary temperatures. The term *mesophil* is often used for microbes that flourish at moderate temperatures. *Psychrophils* may grow in your refrigerator.

If it is necessary to refer to groups of microbes growing between certain temperatures, the best thing is to state the temperatures. Otherwise one becomes bogged in fancy terms. The worst offender in respect of sham precision of this sort is an American opuscule (not mentioned in this book) which professed to teach the fundamentals of bacteriology!

Variance, *n.* A measure of the degree of variation. Methods for the analysis of variance were discovered by Professor (then Dr.) R. A. Fisher.

ANSWER TO QUESTION ON P. 124

About thirty years. This is a quite sufficient degree of precision, as the data are imprecise—"a cupful of soil" is an especially vague measure.

ENVOI, WITH ACKNOWLEDGMENTS

A BOOK is a challenge to specialists. It is necessary to point out that the solutions and other parts of techniques published in this book have often been specially adapted for the beginner, and are not necessarily those used at Rothamsted. The mineral-salts solution suggested as the basis of a medium for " counting " microbes by the plate method is *not* that used by me for exact work of that kind; it is a solution especially suited for the growing of cellulose-decomposing bacteria !

The conception of the book is wholly mine, but the information in it was not. I often wonder how it is that anyone manages or dares to write a factual book, about anything except the narrowest speciality, unless he has the assistance of the type and class which is enjoyed by members of the Rothamsted staff, and in this instance by me: whereby colleagues possessing wide and diverse knowledge give their aid freely and as a matter of course. The feeblest member of the consociation is the author who attempts to write down an account of knowledge which is not his own.

For any mistakes I must be held personally responsible.

I tender my grateful thanks to all who have helped with facts made use of in this book. In addition to those whose help has been acknowledged in the text, I wish to thank the Public Relations Department of the British Post Office for supplying the postal data given on p. 59; also various colleagues at Rothamsted for much help in their specialities: especially Dr. H. G. Thornton, Head of the Department of Bacteriology, for assistance in parts of the book and for drawing Fig. 11; to Mr. S. D. Garrett, for assistance with the thorny subject of fungi; to Dr. S. H. Harper for assisting with the looking-glass chemistry; to Mr. H. K. Chen (Chen

Hwa-Kuei) for facts about some Chinese microbes; and finally to Mr. R. N. P. Luddington, who gave substantial help with the statistical analyses, without which the book (like present-day biological work in general) would have lost much of its force.

References have been given in a less contracted form than that of the *World List of Scientific Periodicals*. At the end of each reference is added in square brackets the serial number of each periodical in the second (1934) edition of the *World List*. The *World List* may be seen at any good public library (at least in Britain), and will inform the reader which libraries in Britain stock each periodical.

I do not wish to discourage earnest enquirers from writing to me about matters touched on in this book, but I may point out that many agencies for the dissemination of information are freely at the disposal of the public, and are only too glad to be given the opportunity of being useful. Farmers in Britain should consult their County Agricultural Organizer or similar authority; elsewhere, the local agricultural experiment station. I have no knowledge about remedies for disease, and if you seek such, you must consult your Ministry, Board, or Department, of Agriculture (about plant disease), your veterinarian, or your family doctor.

I hope that specialists will not put me under the disagreeable obligation of having to refuse them reprints. Students and specialists may be reminded of the extensive facilities offered willingly by the various Imperial Bureaux of Agricultural Science, including the supply of information on specific points, and the provision of abstracts from scientific periodicals appearing in any language. For example, the Imperial Bureau of Soil Science (address: Harpenden, Herts., England) will gladly tell you about itself, and what it does, and will indicate what the other Bureaux do, and where they are, and will either tell you,

or tell you where you can find out, anything else in reason about any aspect of agricultural science, whether you live under the British flag or not. You can also submit enquiries to the Headquarters of the Bureaux, 2 Queen Anne's Gate Buildings, London, S.W.1.

Will anyone still wishing to write to me, please note that the correct address of the institution known for short as "Rothamsted" is below.

Yours cordially,
HUGH NICOL.

ROTHAMSTED EXPERIMENTAL STATION,
HARPENDEN, HERTS,
ENGLAND.

November, 1938.

INDEX

Note.—Merely incidental mentions, such as that of Pasteur on p. 221, and of the microbial species on p. 72 and p. 167, are not included below. When a page-number is enclosed in brackets, the subject, but not necessarily the name or word, is to be found on the page given. An asterisk * denotes that the idea is also to be found in the Glossary, pp. 234–9. Roman numerals refer to the gravure plates.

A

actinomyces (actinomycetes), 35, 111, 115–17, 208
" Adco," 19, 153–4
agar (agar-agar), 39, I
agave plant, 131
aggregation of bacteria, 15, 33
air, filtration of, 123
A.I.V. process, 129
Akron, 175
Alexander the Great, 197
algæ, 29, 37, 85, 161, 167, 213
Alice :
in Wonderland, 16, 21, 79
through the Looking-glass, 11, 16, 46, 76, 88, 115, 150, 171, 234
Allen & Hanburys, Ltd., VIA
ammonia, formation of, 145, 160
ammonia, sulphate of, (148), 154, 157
ammonifiers, 145, 160
amœbæ, 29
analysis, factorial, 95, 224, 225
— microbiological, 98–103
— statistical,* 95, 117–18, 120, 223–5
Ant, river, 190
antiseptics,* use of, 122, 125 *seq.*
apple juice, 126
Applied Mycology and Bacteriology, 44
" artificial farmyard manure," 19
asepsis,* 122, 125–6, VII
Asia, 126, 130
associations, macrobial, 165–6
— microbial, 29, 33, (41), 159 *seq.*
authority, value of, 226–30
averages, dubiety of, 94, 118
Azotobacter, 89–103, 146, 161, 164, 189–96, 218, 236

B

bacteria, 31, 111, 167–9, I, II, IV
bacteria, aggregation of, (13), 33, (163)
— ammonifying, 145, 160
— cellulose-decomposing, 111, IV
bacteria, edible, 213
— nitrate-production by, 160
— numbers of, in soil, 11, 115, *seq.*, 142–4
Bacteriology, Conn, 41
Bacteriology, Tanner, 43
bacteriology, books on, 43–5
— of disinfectants, 79 *seq.*, 114–5
Bacterium prodigiosum, 194–5, 197
ballistics, 205–6
Bangkok, 196
Barger, G., 212
Barritt, N. W., 167
basic slag, 152, 157
Bayliss, Jessie S., 177
beans, 147, 152
— broad, VIB
Beijerinck, M. W., 215–18, 237
Bellani, A., 201
Bénau, O. A., 211
Berlin, 200
Bizio, B., 200–2
Bligh, S. M., 32
books, lists of, 43, 75, 140, 158, 187, 214, 217
boron, 60–1
Bradley, R., 173
Breconshire, 32
Breed, Margaret E., 200
Breed, R. S., 200
broad beans, VIB
Broads, Norfolk, 188
broom-rape, 22
Builth Wells, 32
Buller, A. H. R., 203
Bure, River, 190
Burgerstein, A., 158
Burgess, R., 11
Buxton, A., 190, 191

C

cabbage, pickled, 127
Canada, 205–6
canals, 189, 195, 218, 225
canned foods : canning, 71, 126, 137
carriers (of infection), 137
catalysts, 21

INDEX

Cattle Fodder and Human Nutrition, 129
cellars, 35
cellulose, decomposition of, 141, 161, IV
Ceylon, 163
chalk (chalking), 92, 114, I
Charon, 43
cheese, 126–8, 168
Chen, H. K., 212, 213, 240
Chesterton, G. K., 139, 220
Chet, river, 190
Chinese microbes, 163, 212–13, 241
Chladothrix, 33, 36, 167
chlorine, 42
chlorophyll, 21, 179
chocolate manufacture, 136
cider, 126
Clippesby, 190
clotting, 70, 73
clover, 147, 157
cocci, 28, 30–32
colonies (colonial growth), 13, 49, 111–16, 142, 171, I
Colorado, 175 *seq.*
composts, 19, 146, 151, 153 *seq.*, 168
conditioning of air, 123
Conn, H. H., 44
Conn, H. W., 44
consociation, 166, 240
cooking, 137–8
Coprinus, 111
cresols,* (80), 144
cresylic acid, 80
Crowther, J. G., 37, 43
Croydon, 61
Crump, Lettice M., 228
cucumbers, 107, 128
Cutler, D. W., 228
cyanamide, 156
cycles, natural, 17, 146, 159

D

De, P. K., 38
Delft, 189, 217, 218
Design and Analysis of Factorial Experiments, The, 221
Design of Experiments, The, 219
design of experiments, 93, 219, 221, 225
diatoms, 37, 39, 164, 188
disinfectants,* (122), 125 *seq.*
— for growing of microbes, 76, 80 *seq.*
— in the home, 133 *seq.*
ditch-water, 101, (189), 191, 194, (218)
dividends, 232–3
division, 33–5, 46–50
Doctor's Dilemma, The, 57
Documents in the Case, The, 187
dodder, 22
drains, house, 133–4
— land, 101, 189, 190, 191, 226
Drummond, J. C., 126–7, 139
dust (dusting), 137

E

Ehrenberg, C. R., 200
elective culture methods, 108, 122
electric signs, 220
Ellis, A. F., 131
enzymes, 73–5
epidemics, 56, 199
— in Berlin, 200
— Croydon, 61
— France, 200
— Legnaro, 201
— Tyre, 197
ergot, 211
Ergot and Ergotism, 212
Erlenmeyer flask, 65
evolution, 24, 46, (51)
examiners, 219–20
experience, value of, (95), 226
experiment, meaning of, (219), 222–4, 227
experimentation, factorial, 93, (219), 224–5
experiments, design of, 93, 219, 224, 225

F

factorial experiments, 93, 219, 224–5
fairy rings, 173
fares, 233
Fascism, 219, 229
fermentation, 70–4, 107, 125–31
fermented milks, 72, 129–30
ferments, 70–4
filamentous growth, 33, 163
filtration of air, 123
— of liquids, 125–26, 161
Finland, 129
fish ponds, 133
fish, salted, 108, 127–8
Fisher, R. A., 219, 228, 230, 239
fixation of nitrogen (*see* nitrogen)
— odour, 210
flasks, 65
flies, 165
flour, 137
fluctuations of bacterial numbers, 119, 228
Fred, E. B., 44
Freshwater Biological Association, 196
fruit juice, 126
fungi, 21, 36, Fig. 11, 113, (115), 167, 168, 173, 203, (III); *see also* yeasts

G

Galloway, L. D., 41
Gangulee, N., 32

INDEX

gardening, 150, 173
Gardner, A. G., 44
Garrett, S. G., 240
Gärungssarcinen, *Die*, 217
gelatine, 194–15
Gilbert, J. H., 174, 177
"ginger-beer plant," 33, 162, 164
glue, how to use, 109
gold standard, 231
Golding, L., 199
Goodsir, 215, 216
Gorbach, G., 195
Goslings, N., 215
Grain and Chaff from an English Manor, 159
"*graines vivantes*," 162-3
grass, 154, 157, 173–9
Gray, P. H. H., 86
growth, microbial, 49

H

hair-powder, 210
"hair vegetable," 213
ham, 127
Hampton, F. A., 208
Harpenden, 191, 194 (*see also* Rothamsted)
Harper, S. H., 240
Harris tweed, 211
hay, self-heating of, 52 *seq.*
Herbstmilch, 129
honey, 138
hop manure, 156
Horsey, 190, 194–5, II
host (general), 52, 56
— plant, 32, 52, (147), (164)
host, sacred, 197–9
humus, 84, 151–5

I

"Iceland moss," 40, 211
"illth," 61
immortality of microbes, 46
Imperial Bureaux, 224, 230, 241-2
infants' food, 139
infection thread, 33, (161)
Intelligent Woman's Guide, The, 219
ironing, 137
Iterson, G. van, 217

J

jam, 138
Japan, 213
"Jerusalem bees," 162
Jewish Problem, The, 199
Jews, 199
Jong, L. E. den Dooren de, 217
juice, fruit, 126

K

Keeble, F., 62
"keeping" of foods, 126, 137
kefir, 162
Kelly, C. D., 168
Kenora, 205
Kieselguhr, 40
klongs, 196
Kluyver, A. J., 189, 191, 192, 217, 218
konibu-cha, 163
kumiss, 130, 162

L

lactic acid, (66–7), 68, 72, (130), (160), 184
Lanks, E. C., 131
Large, E. C., 22
Lawes, J. B., 171, 177
lawns, 157
Legnaro, 201
legumes (leguminous plants), 31-3, 147, V, VI
lemony scents, 210
Lessons on Soil, 64, 75
lichens, 40, 209–11
Limburger cheese, 128, 168
lime (liming), 92, 97
Lloyd, 205
Lohnis, F., 44
London Passenger Transport Board, 232–3
London postal districts, 59
Lucerne, 147, VIa
Lucian, 197
Luddington, R. N. P., 211

M

macrobes, 128, 165
maguey, 131
Maret, Ethel, 214
mammals, 12, 51
Marchand, A. K. von, 206
Marshall, C. E., 160
Marshall Ward, H., 164
Mason, J., 141
Massee, 205
Maw, S., Son and Sons, Ltd., VII
Mayer, H. D., 163, 164
Merlino, C. P., 200, 201
Mexicans, 131
microbe, definition of, 12
Microbes and Ultra-Microbes, 44
micrococci, 30, 167, (IV)
micron, 28, (142)
milk as food for microbes, 61, 138
— condensed, 137
— fermented, 129, 162
— "fizz," 130
— pasteurized, 138–40
"miraculous bacillus," 184, 197, II
mixed cultures, 160 *seq.*

INDEX

molasses, 128
Montargis, 196
" moss," 33, 40, 210, 211
mousse de chêne, 209
mushrooms, 21, 173, 187
mycelium, 23–6, 35, 174–8
Mysore, 21

N

naphthalene, 145
Nauru, 131
Nebraska, 205
Nicol, Hugh, 108, (125), 130
nicotine, 184
Nijhoff, Martinus, N.V., 217
nitrate (of potash, and soda), 127, 152
— produced by bacteria, 145
nitrochalk, 154
nitrogen, biological fixation of, 38, 96, 102, 116, 148, 161, 189, 234, V, VI
nitrogenous compounds:
  decomposition of, 145
  synthesis of (*see* nitrogen, biological fixation)
nitrogenous manures, 148, 152, 154, 156–8
no-bacteriology, 116, 124
nodules* (and nodule bacteria), 31–3, 51, 147, 157, 164, V, VIB
Norfolk Broads, 188
nucleus, 24, 39

O

Oby Mill, 190
Ocean Island and Nauru, 131
odour, "fixation" of, 210
— in decomposition, 208
— of actinomycetes, 35, 208
— sanctity, 208
— soil, 35
Okada, Y., 213
opinion, value of, 43, 95, 226 *seq.*
optical rotation, (187)
Opuntia sp., 163
Otomi Indians, 131
Outline of the Universe, An, 37, 43

P

*p*H, 218
Padua, 201
parasitism,* 22–3, 157
Park Grass meadow, 166, (174), 177
Passfields, the, 229
Pasteur, L., 185
pasteurization, 138
pear, prickly, 163
peas, 147, 152
Penicillium, 23, 25, 26, 36, 186
percentages, 94, 231–3
perfumes, 21, 135, 208–11
permanganate, 155
phenol(s),* 80, 144
phosphate, 89 *seq.*, (157)
— deficiency, 96
Piemeisel, R. L., 175–9
Pitra, 206
planning, 226
plating (cultures), 109–17, 142
pollen, 12
postal districts, London, 59
potash deficiency, 96
— nitrate of, 152
potassium salts, 89, 97, (155), 191
prickly pear, 163
protozoa, 27, 44, 48, 146, 164, 167
pulque, 131, 163
putrefaction, 70

R

railways, 220–1
Ramsbottom, J., 132, 163, 212, 213, 214
ratio, idea of, 231–3
Reenen, W. V. van, 189, 191
refrigerators, 137, 239
replication, 82, 93, (94), (106), 117
Researches on Fungi, 203
resistance (to infection), 57–62, 165–6
restaurants, 136
rice-fields, 38, 164, 202
Rippel, A., 45
roads, 220–1, 225, 227
Rocking-horse Fly, 16
Rolfe, F. W., 43, 162, 173, 212
Rolfe, R. T., 43, 162, 173, 212
Romance of the Fungus World, The, 43, (162), (173), 212
root, idea of, 37
— invasion of legume, 147
— nodules on soy bean, V
— utility to plant, 37
rotation, optical, (187)
Rothamsted Experimental Station, 19, 20, 141, 174, 177, 188, 192, 209, 228–9, 240, 242
Royal Commissions, 225–6
Royston, H. R., 43
Russell, E. J., 45, 64, 75

S

St. Olaves, 188, 190
salt, common, 107, 127
saltpetre, 127
sanctity, odour of, 208
sandalwood, 21
Sandon, H., 228
saprophytism, 23, 144, 156
sarcinae, 30, 35, 215
Savory, A. H., 159
Sayers, Dorothy L., 187

INDEX

Scent of Flowers and Leaves, 208
Scheurlen, 199
Serrati, S., 202
Serratia cutirubra, 202
— marcescens, (191–5), 200, 202, (II)
— salinaria, 200
Sette, 202
sewage, 48, 164
sexuality, 35, 39, 47
Shantz, H. L., 175–9
Shaw, G. B., 57, 219
silage, 127–9, 218
Smith, N. R., 228
soil bacteriology, 44, 141 *seq.*, VIII
— as source of microbes, 64, 80
— microbiology, 11, 141
— odour of, 35, 209
Soil Conditions and Plant Growth, 45
soya (soy) beans, 130, V
species,* 31, 33
Sphærobolus, 203
spores, bacterial, 30, 31
— fungal, 24
starter, 160
starved land, 173
sterile, meaning of, 122, 125, 150
strains,* (148), (192), 195, VIA
Styx, 43
sub-microbes, 42, (41)
Sugar in the Air, 22
Suringar, 215, 216
swede turnip, 60

T

Tanner, F. W., 43, 138, 140
tarhô, 129
tartaric acid, 184
tea, fermented, 163
teaching of science, 230
Teinturerie Domestique, La, 214
temperature, effect of, 48, 51, 237
Tengu, 213
Textbook of Agricultural Bacteriology, 44
Thornton, H. G., 32, 86, 141, 178, 192, 228, IV, VIB
thermophils,* 51
Thurne, River, 190
tibi, 163
tinned foods, 74, 126, 137
toadstools, 24, 173, 175, III
to-fa-tsai, 213
toilets, 136
tong-chong-ha-cho, 212
tramways, 227
transport, 202, 220, (225–7), 229
Transport Board, London, 232–3
treacle, 128
Treviso, 202
Tripoli powder, 40
Tyre, 197

U

ultra-microbes, 42, 41
United States, (72), 175 *seq.*, 205–7, 212, 228
Unity of Life, The, 43

V

variance,* 117–18
vascular strand, VIB
Vaughan, R., 160
Vegetable Dyes, 214
Venice, 196
Verzamelde Geschriften, 217
vibrios, 30, IV
Virtanen, A. I., 129
virus, 15, 42

W

Walker, Leva B., 205–6
Ward, H. Marshall, 164
Warington, R., 171, 177
water, filtration of, 164
— microbiology of, 100, 169, 188 *seq.*
Webb, Beatrice, 229
Webb, Sidney, 229
Wells, H. G., 229, 230
wine, 126, 130
Winogradsky, S., 234
Worden, S., 228
World Encyclopædia, 229
World List, 241
Wroxham, 188, 190

Y

Yare, River, 190
Yates, F., 224
"Y.B.," 33
yeasts, 24, 47, 101 *seq.*, 163, 167, 212

Z

Zoogalactina, 202
Zoochlorellæ, 29

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Emergency Landing

My husband pushed back the cockpit cover, put on his helmet and goggles, heightened the seat for better visibility, and leaned forward to look out. Here we are again, I thought, recognizing by this familiar buckling-on-of-armour that the fight had begun.

Here we are again. We seem to be always here, I thought, fear opening for me a long corridor of similar times and making them all one long fight with fog. There was the time over the Alleghanies in the *Falcon*: slicing over the tops of those pine trees, now on one wing, now on another. Then we found the river down below the mist cutting a gully through the mountains. We followed it and came out to safety. But then with a *Falcon* it wasn't so dangerous—not so fast. The *Sirius* now—— (He was headed for that peak in the clouds. How did he know there was not a lower peak—a shoal, just covered by this high tide of fog—that might trip us up as we skimmed across the surface?) Then, there had been that time over the pass of the San Bernardino Mountains. "Ceiling very low," read the weather report. "What does 'very low' mean?" my husband had asked. "Two or three hundred feet?"

"It means nothing at all," laughed the weather man. "You'd better stay here."

"Oh, we'll go see what it looks like—may come back—may find a hole." And up we had climbed until we were face to face with the giants, snow-streaked, a

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Emergency Landing
(continued)

bright fog sitting on their shoulders. But that time, too, finally we struck a stream and followed it—a beautiful stream with fields to land in and green orchards and houses. It carried us along and spilled us out into the broad valley of San Bernardino. I had it like a ribbon in my hand all through the fog. I held on to it and it led us out.

Here there was nothing to hold on to—nothing, unless it were the sky. The sun still shone. My husband motioned me to reel in the antenna. Emergency landing, that meant. I buckled my belt tighter. We were circling the giant's head now, getting impudently nearer and nearer. Down, down, we were gliding down now, the engine throttled, wisps of fog temporarily blinding us as we descended. I was losing the sky. I did not want to let go until I could grasp something below. Down the sides of the mountain one could see a strip of water gleaming, harebell-blue. We were diving toward it. Down, down—the sky was gone. The sea! Hold on to the sea—that little patch of blue. Oh, the sea was gone too. We were blind—and still going down—oh, God!—we'll hit the mountain! A wave of fear like terrific pain swept over me, shrivelling to blackened ashes the meaningless words "courage"—"pride"—"control." Then a lurch, the engine roared on again, and a sickening roller-coaster up. Up, up, up. I felt myself gasping to get up, like a drowning man. There—the sky was blue above—the sky and the sun! Courage flowed back in my veins, a warm, pounding stream. Thank God, there is the sky. Hold on to it with both hands. Let it pull you up. Oh, let us stay here, I thought, up in this clear bright world of reality, where we can see the sky and feel the sun. Let's never go down.

He is trying it again, like a knife going down the side of a pie tin, fog

CONTINUED